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SOME DEVELOPMENTAL EFFECTS OF X-RAYS IN *DROSOPHILA*

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(With Eleven Text-figures)

If *Drosophila* larvae homozygous for the third chromosome recessive *straw*-3 are treated with X-rays, back-mutations of the *straw*-3 gene to its normal allelomorphs take place in somatic cells, and the cells of the wings give rise to hairs which can be recognized as wild type (Waddington, 1940*b*). In one attempt to follow out this matter in more detail high doses (7000 r. units) were applied. The resulting mortality was, it might be said, 100 %. That is, no flies emerged; but on dissecting the pupa cases, it was found that a considerable proportion had developed up to the hatching stage, but had not been able to free themselves from the pupal integuments. These flies were found to exhibit some rather remarkable deformities, and the experiment has therefore been repeated a number of times. It is the purpose of the present paper to describe in some detail the developmental abnormalities produced by such heavy doses of X-rays. Friesen (1936) originally described some abnormalities (which he termed 'Roentgenmorphoses') produced by slightly smaller doses, and some further notes on such cases have been published by Enzmann & Haskins (1938*a*, *b*) and Haskins & Enzmann (1937). All the abnormalities found by these authors can probably be considered as due to inhibitory actions of X-rays on various developmental processes; but, as will be clear later, the striking feature of the animals to be discussed in the present communication is that in some of them the growth of certain rudiments has been actually stimulated by the radiation. A preliminary communication of the experiments recorded in this paper has been published (Waddington, 1942).

MATERIALS AND METHODS

Several different genotypes were used: wild type, *straw*-3, *dachs*, *split singed*-3 and *morula blistered*-2/*Curly*. Most of the experiments were made with the first three types, but no differences in behaviour were found with any of the genotypes, and the results of all the experiments can therefore be grouped together for description.

Larvae were collected by allowing a number of flies to lay eggs on normal food in 3 × 1 in. vials for a given period, the cultures then being kept at 25° C. until irradiation, after which they were returned to an incubator at the same temperature. Pupae were collected as 'white prepupae' from the sides of culture bottles; as the puparium darkens within 3–4 hr. of its formation, their age was known within those limits.

Irradiation was in all cases with a dose of 7000 r. units. The X-rays were generated at 150 kV. constant potential, 7 mA., and filtered by 0·7 mm. copper, 1·2 mm. aluminium.

The half-value layer was 1·0 mm. copper, corresponding to a mean wave-length of 0·15 A. The intensity was 79 r./min., and the duration of the exposure 89 min.

DESCRIPTION OF RESULTS

The results can be described in groups, according to the age of the larvae or pupae at irradiation.

(1) *Age 27 hr. after puparium formation.* A considerable number of wild-type pupae of this age were irradiated. Mortality was comparatively low, and some of the flies emerged unaided from the pupa cases. No important morphological abnormalities were found, except for the rare absence of a microchaete.

(2) *Age 19 hr. after puparium formation.* Mortality was much higher; none of the flies emerged unaided, and many died before attaining the emergence stage. Those which survived were dissected from the pupa cases. The most marked effect was on the bristles, many of the macro- and microchaetae being absent. In most flies the proportion of microchaetae affected was somewhat larger than that of macrochaetae, and in a few the microchaetae were almost entirely removed while the macrochaetae were present in the full normal number. In nearly all affected hairs, both the hair and its socket were absent, but cases were found both of hairs present with no socket, and sockets with no hair.

A few of the eyes appeared to be slightly rough, but the structural basis of this was not investigated in detail.

(3) *Late larvae and young prepupae.* The largest series of these had been timed from laying, and were aged 105–113 hr. at 25° C.

The bristles were very extensively affected, but the microchaetae were again the most severely damaged (Fig. 1). Only one case was found in which one of the two components was affected alone; in that there was a socket with no hair. Certain abnormalities in the hairs were also found: a few macrochaetae were doubled (e.g. post-verticals in Fig. 1), there was an occasional *forked* or *singed* type, and rather more frequently the T-shaped *shaven* type (Lees & Waddington, 1942).

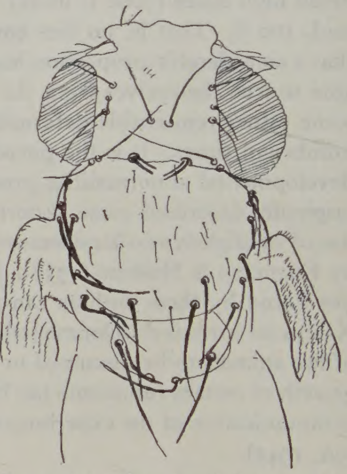

Fig. 1. Adult from irradiation at white prepupal stage.

The anterior-dorsal parts of the eyes were sometimes rough, but this effect was much stronger in some of the younger groups. The wings were highly abnormal. Their shape and size was that characteristic of a 48 hr. pupa (stage P 2*d*), and they had not increased in size to any great extent after the formation of the pupal sheath, since they lay more or less flat within it, without showing the normal folding. Their smallness was clearly due to the death of a considerable number of cells. This could be deduced from two facts: many of the characteristic marginal bristles were missing, so that there were many gaps in the normal row; and, in spite of the small size of the wings, the size of the cells, as judged by the distance between the cell hairs, was not proportionately reduced. The cell hairs were, however, much more variable in size than usual, suggesting that many of the surviving cells were somewhat damaged by the treatment.

Although the wings were small, the pupal wing sheaths were of normal size, and, as has been said, the wings lay more freely than usual within them. These sheaths are, of course, secreted by the wing itself at the time of its greatest inflation, just before true pupation (about 15 hr. after puparium formation). This suggests that the cells do not die until after the inflation period; and this is confirmed by direct examination. In late prepupal wings, no sign of degenerating cells can be found. In wings of the contraction phase of the pupa (stage P2*d*), on the other hand, the whole surface is covered with a layer of feebly staining jelly-like debris, many necrotic and pycnotic nuclei can be seen in the epithelium, while the vein lacunae, which are much wider than usual, are nearly filled with granular lumps of dead material. The death of the cells is therefore postponed for some hours after the irradiation.

After the application of somewhat smaller doses of irradiation in other organisms, it has commonly been found that death of the cells takes place at the next division following the exposure to the X-rays. In *Drosophila* the cells of the wing are very unfavourable histological material, and the occurrence of a division phase at the time of contraction has not been directly demonstrated. Such a period of division is, however, known to occur in other insects at a comparable stage, and it is most probable (cf. Waddington, 1940*a*) that *Drosophila* behaves in the same way. If that is the case, the postponement of necrosis from the time of irradiation till the contraction phase would fall into line with the phenomena described in other material.

(4) *Late larvae, age* 90–96 *hr. after laying.* Mortality was considerably higher than in the pupal classes. No flies emerged, but enough survived to the emergence stage for their development to be followed.

The bristle effects were still very strong. In most flies both macro- and microchaetae were affected, but in this group the former were more sensitive, and the latter less so than in the older groups (Fig. 2). Again, occasional bristles showed only one of the components affected, and *shaven* and *forked*-like types occurred. In one fly extra dorso-central bristles were present, but the significance of this is doubtful, since the number of these bristles is somewhat variable in normal wild-type animals.

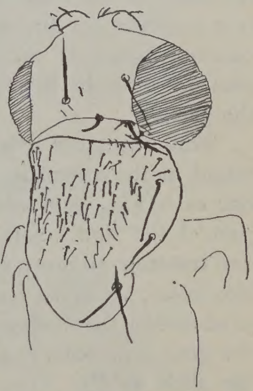

Fig. 2. Adult from irradiation as late larva, aged 90–96 hr.

The eyes were nearly always rough, and sometimes were extremely reduced in size. Their anatomical structure was very varied. In some cases the pigment cells were almost lacking, in others the rhabdomeres were absent, while in others again, patches of retinal tissue appeared to be transformed into post-retinal material. The histology of these different abnormalities has not yet been studied in detail, but it is probable that different components of the eye have slightly different periods of maximum sensitivity, which could only be disentangled with accurate timing: further investigation of this problem is in progress.

A few of the wings were affected as in the last group, but many of them appeared to be fairly normal; owing to the difficulty of expanding them, after dissecting the flies out of the pupa cases, it was impossible to examine them in detail, but there is no doubt that they were of at least approximately normal size. The legs, on the other hand, were very badly crippled. They were bent, frequently very short, and often bloated, the segmentation of the tarsus being sometimes almost completely obliterated. The abnormalities strikingly resembled those found in the mutant '*comb-gap*'. The most important event leading to their formation is a disturbance of the normal process of eversion of the leg rudiment from its peripodial sac in the early prepupa. Any malformations produced by difficulties of eversion tend to become even more exaggerated as pupal development proceeds. The legs undergo the usual process of inflation at the end of the prepupal period, but frequently fail to carry out the subsequent contraction, since their irregular shape, sharp bends and mutual interference, obstructs the flow of body fluid out of them into the main body of the thorax.

(5) *Mid-larvae*, 60–90 *hr. after laying*. Several different age groups falling within this period were separately irradiated, but as they gave essentially the same results they may be treated together. Mortality was always very high, and this is probably the period of maximum sensitivity during larval life. The first notable point is that bristle effects are nearly completely absent in this group; both macro- and microchaetae are formed in full numbers. On the other hand, a new type of effect is found which did not occur in the older animals. This is a tendency for the overgrowth of various regions of the body, which sometimes results in reduplication of the part.

This effect has only rarely been found in the antennae, but there is one case (Fig. 3) in which the basal part of the right arista is thickened and transformed into a somewhat leg-like tissue; the arista resembles those produced by the weakest expression of the gene *aristopedia* (e.g. typically by the allele **ss**$^{a-B}$). This certainly involves some overgrowth, but of course no reduplication.

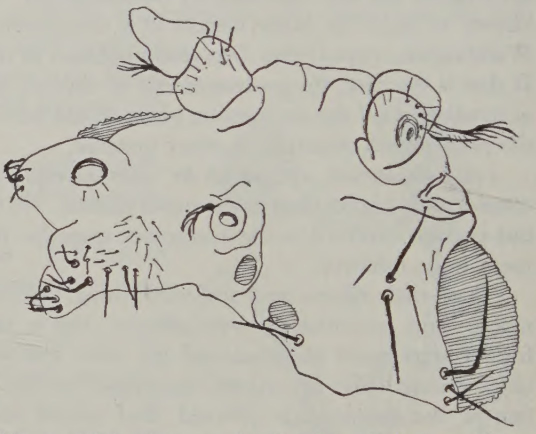

Fig. 3. Adult of *straw*-3, irradiated as mid-larva, aged 72–90 hr. Head, note thickened base or arista at right, extra antenna near ocelli, and basal antennal joint in place of left eye.

The eyes are very constantly affected. In one or two individuals they are very much reduced (on both sides), only a few malformed ommatidia being formed. The histology of these has not been studied. A very much commoner effect, found to some extent in nearly all the remaining individuals, is the formation of a palp protruding from part of the eye surface. In the commonest and 'weakest' form, this is merely a flattish plate of chitin, bearing a few hairs, covering the anterior-dorsal region of the eye (Fig. 4). In other cases the plates are large and protruding, bearing a number of macrochaetae as well as some microchaetae (Fig. 6). In such flies, the size of the eye is not reduced by an area equal to that of the palp, although a certain degree of reduction does sometimes occur. Usually, however, it appears that the eye is of normal size but is displaced downwards, towards the ventral surface of the head. The palp must, at least in part, represent additional material. This is particularly clear in a few cases in which the eye is exceptionally large, with a band of palp-like material running across it from front to rear (Fig. 7).

Here the lower part of the eye is of normal full size, while the part above the palp is additional, and represents a reduplication.

The palp usually shows no very definite resemblance to any particular part of the body; and this is normally the case also for the eye palps sometimes found in *Lobe* flies and regularly produced by the gene *ophthalmopedia*. The suggestion, implied by the latter name, that they represent a malformed leg, must not be taken too seriously. In a few of the X-rayed cases, definite organs can be detected in the palps. In one case (Fig. 7), a flat expanse of palp on the dorsal surface of the head bears an extra ocellus. In another, a large protruding palp on the left eye contains a Johnson's organ, which identifies it as an antenna, while a further palp-like expansion on the same side of the head contains a second similar organ, in this case accompanied by a small plumose spike resembling an arista (Fig. 3).

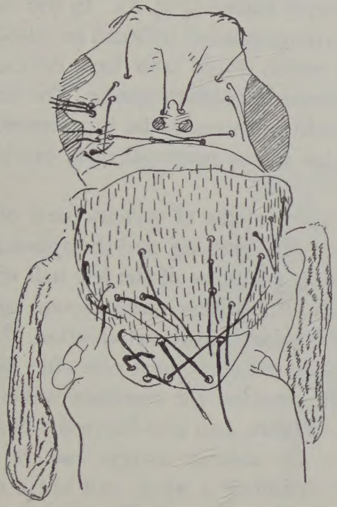

Fig. 4. Adult, from irradiation at 66–72 hr. Note palp in left eye region, duplicated left scutellum.

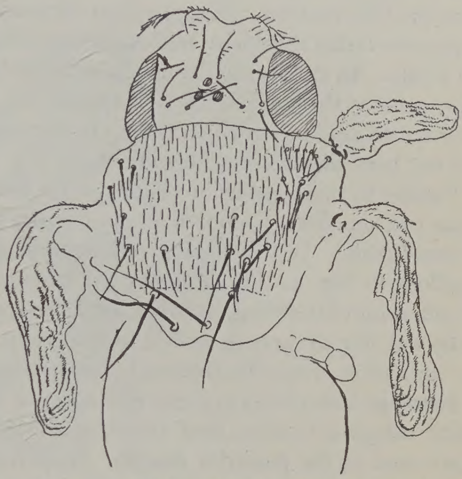

Fig. 5. Adult from irradiation at 66–72 hr. Note duplicated wing region at right.

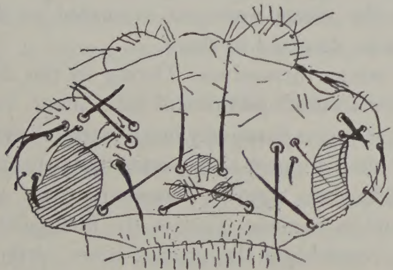

Fig. 6. Adult, from irradiation at 72–90 hr. Head, note palps on both eyes.

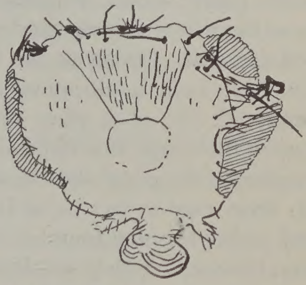

Fig. 7. Adult from irradiation at 72–90 hr. Posterior aspect of head, showing palp above left eye, with extra ocellus, and palp separating right eye into two halves.

The formation of palp-like outgrowths is sufficiently common for one to be able to identify it in sections of earlier stages. Such outgrowths can be traced back to the early prepupa. In sections through the anterior end of the animal at that time, the eye-forming region can be identified by the regular grouping of its cells (see Steinberg, 1941; Pilkington, 1942) at the posterior end of the cephalic imaginal buds; it normally forms a smoothly curved surface which fits snugly against the brain. In the X-rayed animals, however, the central region of this smooth surface is sometimes deeply folded (Fig. 8). In the tissue lining the fold, no trace of the usual pre-ommatidial arrangement of cells can be detected. It appears certain that these folds represent material which would later have developed into a palp. An exactly similar appearance has been found in *ophthalmopedia*. The lateral palps, such as that seen in the left eye of Fig. 4, would presumably be represented by abnormal folding of the edge of the eye-forming region in the prepupal disk, but these have not been detected in the sections.

Passing to more posterior regions of the animal, overgrowths of various parts of the thorax can sometimes be found. They normally produce only swelling, and the appearance of extra bristles. In a few cases, real reduplications may occur, as of the left half of the scutellum in Fig. 4. Fig. 5 shows one example of the interesting phenomenon of the reduplication of the wing, together with a region of thorax around its articulation. Since the fly was, like all those described in this group, dissected out of its pupa case after death, the extra wing cannot be flattened, and its shape and venation are therefore unknown. Its histology is perfectly typical; it is covered with cell hairs, and also bears the characteristic marginal bristles, both the short thick ones of the anterior margin and the thin longer ones of the posterior margin. It is therefore definitely a wing, and not a mere persisting larval spiracle, such as those described by Enzmann & Haskins (1938*b*), although such persisting spiracles have also been seen.

Friesen (1936) found, particularly after irradiations at this stage, a considerable number of cases in which marginal parts of the wing were missing, as in the well-known scalloped mutants. Owing to the fact that the wings were not expanded in the flies described here, such modifications could not be detected in them.

Overgrowth also takes place within the wings themselves. Owing to the difficulty of flattening the wings, it is impossible to give accurate pictures of their shape, but even an inspection of the pupal sheaths will show that comparatively large extra lobes may be formed; these may in fact be so large that they suggest that partial reduplication has occurred, rather like that found in *blot* (Waddington, 1939). A somewhat more peculiar result has been seen both in whole mounts and in sections. That is, the formation by the wing membrane of a thick chitinous layer, resembling the normal body surface, and, like it, bearing fairly large bristles, complete with sockets (Fig. 11). No developmental stages of this are available. The process has not been described in any mutant stocks. It might be supposed to be due to the formation of isolated spheres of wing epithelium, but in fact such structures, at least when they occur in *bloated* (Waddington, 1940*a*), always develop the normal wing histology.

A phenomenon which may perhaps be related to the last is the conversion of the entire wing into a lump with a normal body surface bearing chaetae. This has been seen in several adult flies. It occurs occasionally in certain stocks, for instance, one of *expanded* which was kept at Pasadena, but whether the effect is due to a separate gene, or what its mechanism may be, remains undetermined.

The legs are often extremely irregularly shaped, with the usual *comb-gap*-like features described for the older groups. In most cases it is difficult to make out whether they are increased in size or not, but in one or two cases, clear duplications have been found (Fig. 9). Incomplete fusion of the halves of the thorax is also fairly common in these flies, and is probably due to some irregularity of eversion similar to that involved in the leg malformations. Total lack of eversion of legs or thorax parts may occur.

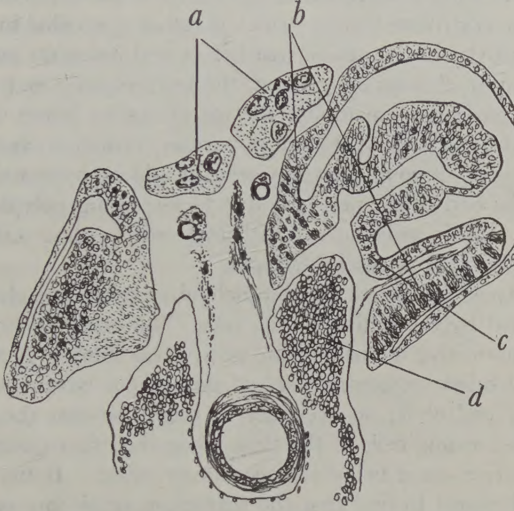

Fig. 8. Early prepupa, transverse section in anterior region. *a*, Weissmann's ring; *b*, eye-forming region of right imaginal bud; *c*, fold in bud, precursor of palp; *d*, right hemisphere of brain.

(6) *Young larvae, aged less than* 60 *hr. after laying.* In larvae X-rayed at ages less than 60 hr., the survivors show progressively less profound modifications of development the younger they were at irradiation, and the flies developed from larvae aged less than 48 hr. at raying were more or less normal.

THE DEVELOPMENTAL MECHANISMS INVOLVED

It will be convenient to begin by discussing separately the mechanisms of the effects of irradiation on the various organs.

(*a*) *Bristles.* Only two cells are concerned in the development of each bristle and socket, and the developmental system is therefore very simple. It is clear from the 27 hr. pupae that this system is not very sensitive to irradiation at that time, although we know (Lees & Waddington, 1942) that the secretion of the hair and socket are by no means complete at that stage. The 19 hr. pupae, which are the next group, exhibit considerable sensitivity. At that time, the hair cells have already divided to form a group of two, but these are still quite small. The decrease in sensitivity between 19 and 27 hr. would be expected (cf. Stadler, 1932) as a consequence of the increasing polyploidy of the cells, which clearly accompanies the growth in size of the nuclei (Lees & Waddington, 1942): it might also be connected with the onset of secretion, but there is no evidence of this.

The sensitivity of the hair cells extends from the 19 hr. pupae back till about 12 hr. before puparium formation (90 hr. larvae). This agrees fairly well with the data of Friesen

(1936). Using somewhat lower doses of irradiation, he found the highest percentages of 'Borstenreduktion' in the groups within 24 hr. before or after puparium formation. It is impossible to make out exactly what was involved in all these cases of bristle reduction, since the one protocol which he provides shows that some of the flies exhibited a lack of many bristles, while in others only a single one of the rather variable head bristles was missing. If the present material were described in the same way, the sensitive period of the bristles would certainly be extended farther backwards towards the earlier parts of the larval life. But the most striking feature, which is rather concealed in Friesen's account, is the very high sensitivity of the period just before and just after puparium formation.

It is clear that within this general period, the microchaetae and macrochaetae have different phases of maximum sensitivity, the macrochaetae being on the whole more severely affected in the earlier part, before puparium formation, and the macrochaetae in the later part. This is, of course, exactly what would be expected if the decrease in sensitivity towards the end of the period is due to increasing polyploidy, since there is little doubt that the rapidly growing macrochaetal cells become much more polyploid than the comparatively small microchaetal ones.

The great majority of the effects are complete absences of the chaetal elements, and are presumably caused by the killing of the cells. The occurrence of a period before which the cells are insensitive might indicate one of two things. It might be, first, that definitely determined bristle cells were present throughout larval life, but only became sensitive towards the end of it; or, secondly, it might be that the cells only become determined as bristle-forming cells at the time when they first exhibit sensitivity, cells knocked out at an earlier stage being replaceable by others. If the former alternative were true we should expect to find that the maximum sensitivity corresponded to the period just before the division into the two components, trichogen and tormogen, from which the bristle and socket arise. This, however, cannot be the case. The sensitivity is still marked at 19 hr. after puparium formation, when the division is known to have taken place; moreover, the division phase can hardly be supposed to last so long as the phase of sensitivity. It seems probable that a pre-division sensitivity, if it occurs in these cells, would only cause a minor variation in the general reaction with which we are dealing. The second alternative given above is therefore to be preferred; and we may take the evidence to indicate that during the early part of larval life the bristle-forming cells are not irrevocably determined, so that if some of the presumptive bristle cells are destroyed, other cells may take their place.

Contributory evidence that the bristles become determined at about 80–90 hr. after laying may be found in the experiments of Plunkett (1926), Goldschmidt (1935), Child (1935), Ives (1935, 1939), and Child, Blanc & Plough (1940) on the temperature effective periods of various bristle-removing genes. In all cases they found that the removal of bristles is not produced by heat treatments administered earlier than about 3½ days after laying. It is, however, not at all clear just what is going on in larvae subjected to heat treatment, whereas one can be fairly certain that at least the primary effect of the irradiation is to kill a certain number of cells. But, whatever may be the modus operandi of heat treatment, its lack of effect in early larval life can be most plausibly explained by the supposition that the bristle-determining process has not occurred at that stage.

It is noteworthy that the X-ray effects very commonly lead to the disappearance of both the bristle and its accompanying socket. This would be only to be expected if the irradiation led to the death of the bristle cell before it had divided into trichogen and tormogen. But in the 19 hr. pupa this division has certainly already occurred. If the effect of the radiation was confined to the killing of individual cells, it is easy to calculate the proportion of cases in which only one, or both, the trichogen and tormogen should be affected. If the proportion of hairs absent was x, and that of sockets absent was y, it should only be in the proportion xy of cases that both a hair and its own socket were missing. But actually both components are usually affected. The proportion of isolated hairs or sockets is highest in the 19 hr. pupae, but even there it is less than that of cases in which both structures are missing. For instance, in one 19 hr. pupa there were fifty-seven microchaetae with both elements, seven isolated sockets and three isolated hairs on the dorsal surface of the thorax in a region in which the total normal number of microchaetae is about 200; in another fly the figures were fifty-two complete microchaetae, sixteen isolated sockets and seven isolated hairs. Thus the failure of both elements had occurred much more frequently than that of only one of them. This must indicate that the killing of one component inhibits the development of the other, even if the latter remains potentially viable. It is probably significant that isolated sockets are commoner than isolated hairs, since the development of the socket, which lies on the surface, is, one would imagine, less inhibited by the death of the underlying trichogen than the latter would be by the death of the tormogen.

The exact time of division into trichogen and tormogen has not been determined histologically, owing to the difficulty of identifying the hair cells before their growth phase. The meagre evidence that was available suggested to Lees & Waddington (1942) that it occurred about 15 hr. after puparium formation. It might be possible to date the division more accurately by the occurrence of isolated hairs or sockets in these irradiated flies, since, if the above explanation is all that is involved, these should not occur in flies irradiated before the division. The isolated components are, however, found, though very rarely, even in the youngest groups which gave any bristle effects at all. The same fact can be unearthed from Friesen's account, since his 'Reduktion von *o*-Typus' can certainly be interpreted as the formation of a socket without a hair. This might be taken to indicate that the division occurs almost immediately after the cell is determined as a bristle cell; the comparative rarity of the phenomenon in the young groups would then be attributed to the greater time available for a dead cell to inhibit the development of its surviving partner. But an alternative explanation is possible. It is known that irradiation may produce chromosome rearrangements of such a kind that a subsequent division leads to the formation of two daughter cells of differing genetic constitution, one of which may be viable while the other is not; and the rare early examples of isolated components may be due to a process of this kind. The attempt to date the time of division does not, therefore, lead to a conclusive result.

(*b*) *Legs.* The most common effect on the legs is to cause a crumpling and bending which can, as was stated above, be traced back to irregularities in the eversion of the leg rudiment from its peripodial sac. Similar malformations are well known in mutant races. They are probably exemplified in *crippled*, and I have investigated their development in *comb-gap*. In the latter, the crippling is accompanied by an irregularly increased growth. This is probably also true of the X-ray malformations induced in larvae aged 90 hr. or less, although there the legs never attain the enormous size sometimes found in *comb-gap*. It is more doubtful, however, whether overgrowth can have taken place in the short interval between irradiation and eversion in the flies issuing from treated larvae aged more than 90 hr.; and in these the legs, although showing the same kind of malformations, did not seem to be any larger than normal. The disturbance of eversion was in these cases probably brought about not by increase in size but rather by decrease, consequent on the killing of some of the cells. It is clear that such disturbances can be fairly easily caused by a number of agencies, since even normal untreated animals which develop in unhealthy conditions may have one or more malformed legs of the typical *comb-gap* type.

In a few cases among the early irradiated animals, overgrowth has been followed by duplication; and there are some cases of atypical histogenesis, e.g. the tarsal claw in a non-terminal position in Fig. 10. These phenomena are discussed in more detail later.

(*c*) *Eyes*. The later irradiations produced a roughening of the eye surface. The histology of these eyes has not yet been investigated in detail, but the roughness is presumably caused by the death of some cells, which would lead to the development of irregular ommatidia. It should be noted that in some cases only part of the eye is affected, and when this is so, it is the anterior-dorsal border which seems most sensitive; this is the same region as that favoured for palp formation in the earlier irradiated groups.

In these earlier groups, the formation of palps is extremely frequent. The evidence summarized above (p. 106) shows that the palps are sometimes derived from regions of the eye rudiment which have been abnormally folded; and that they are often accompanied by increases in size. It is simplest to assume that the formation of a palp is in all cases preceded, and presumably caused, by an atypical folding. Overgrowth would then be one condition which would naturally lead to some abnormality in folding and would thus tend to produce palps.

The formation of reduplicated eyes, such as that in Fig. 7, is not difficult to understand in the light of the above discussion. Overgrowth of the eye region has certainly taken place, and part of it, in a strip across the whole width, was presumably folded inwards so that it developed as body surface, thus separating the lower, more or less full-sized, ommatidial region from the upper smaller one.

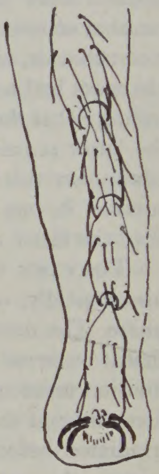

Fig. 9. Adult, from irradiation at 66–72 hr. Tarsus inside pupal sheath. Note doubleness of terminal segment, and double claw.

The dorsal palps, such as those of Fig. 4, may perhaps be attributed entirely to extra material formed by overgrowth, but in many cases, such as that of Figs. 3 and 6, there has certainly been a conversion of presumptive eye material into palp. The majority of the palps develop as rather unspecific 'body surface' bearing some chaetae. The minimum hypothesis is to suppose that, once ommatidial development has been inhibited, the development of body surface proceeds without needing any specific stimulus; and this is supported by the fact that similar tissue can be formed on the wing (p. 106). But in a few cases more definite organs have been formed, ocelli or antennae of various degrees of completeness. Some definite stimulus, or at least some definite level on a gradient, must be necessary to produce these specific structures. The exact nature of these stimuli cannot yet be determined, but the general phenomenon of atypical histogenesis is discussed again later.

(*d*) *The thorax.* The main effect noticeable on the thorax, apart from disturbances of eversion, is an increase in the size of some parts. This may produce a swelling, such as that of the right shoulder of Fig. 4. Less often, it causes a reduplication, such as that of the left half of the scutellum in the same figure or of the wing-bearing region in Fig. 5. The conditions under which overgrowth of imaginal tissue causes reduplication in *Drosophila* are not at all clear. Enormous overgrowth of some of the imaginal buds is found in some combinations of mutants, for instance in *dachsous-dachs* or *dachsous-fourjointed* (Waddington, 1942). In both these, we tend to find reduplicated antennae, often combined with the absence of the corresponding eyes, while the thorax is produced into very large forwardly-reaching shoulders, like the right of Fig. 4 but much larger. The antennal effect is due to an overgrowth in the antennal region of the cephalic disk, and regularly leads to duplication; the thorax effect, which is caused by an overgrowth of the dorsal mesothoracic bud, does not lead to duplication, and no cases of extra wings have been observed. This appears to indicate that the antenna is a more closely organized morphogenetic unit than the thorax, and cannot easily be increased in size relative to the rest of the organism; if an unduly large region is determined as antenna, it can only develop into two antenna. The thorax, on the other hand, appears to be more capable of modification to incorporate extra material. Similar differences in the 'extensibility' of individuation fields are well known in amphibian development. Thus, for instance, the main dorsal axis can easily be caused to incorporate additional tissue, whereas the limbs show a much stronger tendency to become reduplicated.

This modification of the thorax does not occur so regularly in the irradiated flies as in the mutant types such as *combgap-fourjointed*; in the former we find duplications of the wing and thorax regions which do not occur in the latter. The explanation of this difference is obscure: it may be connected with the general unhealthy condition of the tissue caused by the irradiation.

(*e*) *Wings.* The gross anatomy of the wings cannot be made out in detail in these flies dissected out of the pupa cases. Two phenomena, however, require notice. One is the formation, by wing epithelium, of typical body surface, as in Fig. 11. This has never been described in any mutant. It is an example of the general phenomenon of atypical histogenesis, which is discussed later. The conversion of the entire wing into a lump of body surface occurs sporadically in some mutant stocks, but nothing is known of its mechanism; one might guess that it is connected with a failure of the folding of the wing into the cavity of the bud.

The second phenomenon is the formation of extra outgrowths of the wing. This is, of course, another example of overgrowth, which is also discussed in general below. The outgrowths seem, as far as one can judge them in their folded state, to be duplications of parts of the wing. Duplications, springing from the proximal end of the posterior margin of the wing, are characteristic of the mutant *blot*. The condition in the imaginal buds of this mutant have not been investigated, but one can hardly doubt that the duplication supervenes on an overgrowth. Morgan, Schultz & Curry (1940) have pointed out that the mutant *blot* is located very near the breakage points of the chromosome

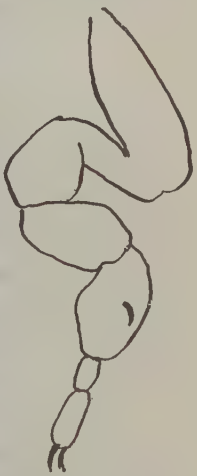

Fig. 10. Adult, from irradiation at 72–90 hr. Leg, showing bent and inflated shape. Note extra tarsal claw on proximal joint of tarsus.

rearrangement *Xasta*, which produces the phenotypic effect of an apical incision into the wing. This apical incision is due to an abnormal position of the wing fold in the imaginal bud (Waddington, 1939, 1940*a*), and the above authors remarked that the very different developmental processes leading to the *blot* and *Xasta* effects made it difficult to consider the latter as a position-effect modification of the former. This difficulty may, however, be mitigated if one considers two facts; we have evidence in the data presented here that overgrowth may lead to reduplication, and we know, from mutants such as *vestigial*, that undergrowth may cause shifts in the position of the wing fold. It is only necessary to reverse the latter datum, and assume that overgrowth may also sometimes lead to shifts of the wing fold, to reduce both *Xasta* and *blot* to similar primary effects, namely, increases in growth. The question of whether, in a given case, an overgrowth leads to a reduplication of part of the wing, or to a shift in the wing fold, is presumably dependent on the exact quantitative relations.

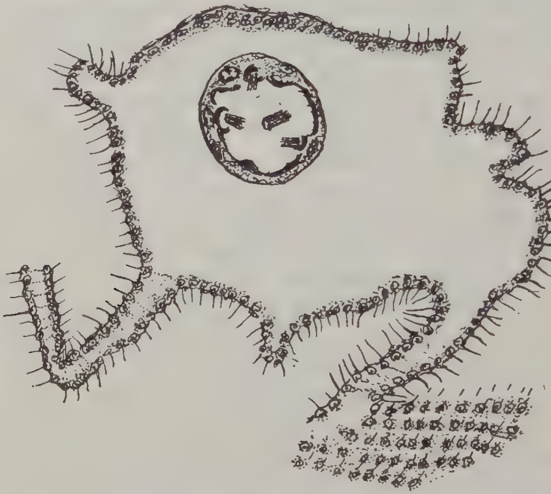

Fig. 11. Pupa shortly before emergence, section through wing. At right below part of the wing membrane is cut tangentially, above it is cut transversely and there is a vesicle of normal body-surface bearing large bristles.

DISCUSSION

The effects produced by the irradiations in the later stages are of a kind which might have been expected. They seem to be easily explicable in terms of the killing of individual cells, modified to a rather slight extent by secondary effects due to the interaction of cells in development, as exemplified in the unduly high proportion in which both components of the hairs are removed. The effects of the earlier irradiations are much more peculiar and provide a considerable amount of quite new information about the developmental mechanisms of *Drosophila*.

The first point which must be mentioned is that the irradiations of earlier stages produce morphological changes which affect quite large groups of cells, comprising considerable areas of tissue. These areas are much too large to be derived, in the time available, from successive divisions of a single original cell. Patches of altered tissue formed in the latter way are well known in experiments in which somatic mutations have been produced by X-rays. With doses of a few hundred to one or two thousand r. units, patches derived from a single somatically mutated cell never include more than a few score adult cells even when the irradiation takes place shortly before the hatching of the larva from the egg. With the much later irradiations described here, the patches should be still smaller. Since there is definite evidence of increased growth in the surviving cells in these experiments it is possible to ask whether this increase might have been sufficient to increase the size of the altered patch up to that found; but the answer must almost certainly be in the negative. Moreover, the alterations produced (duplications of organs, conversion of one tissue into another) are not of a kind which can easily be attributed to mutations of individual genes; they are much more akin to the normal developmental changes which are carried out by the genotype as a whole. The explanation of these 'mass effects' must be sought along other lines than the multiplication of a single original affected cell.

On the other hand, there is no doubt that the immediate effects of X-rays in living tissue are to cause ionizations, each of which occupies a very small space, and affects only a single cell. The production of the same developmental change in all members of a large group of cells can only be attributed to mutual interactions of the cells subsequent to the original X-ray effects. The injury caused by the irradiation must, in fact, produce changes in the physical conditions within the mass of cells, or the release of substances capable of diffusing throughout the mass, and it is to such pervasive secondary effects that we must look for the immediate causes of the alterations in development. Since we know, both from many other investigations and also from the effects of later irradiations on the bristles, that large doses of X-rays cause the death of a considerable number of cells, it is simplest to imagine that it is the dead or necrotic cells which release the diffusing morphogenetic substances or cause the general alteration in physical conditions; but it is of course possible that one of the immediate effects of the radiation is to alter the metabolism of the surviving cells in such a way as to bring about the secretion of an abnormal morphogenetic substance.

The detailed mechanism by which the immediate effect on individual cells becomes transformed into the final alteration of the development of a large mass of tissue must remain for further investigation. But the mere fact that such alterations can be produced at these stages is of considerable importance for our understanding of the mechanics of development in *Drosophila*. The changes are of three main kinds: alterations in histogenesis, such as the production of eye palps; increases in growth; and the production of duplications. As was made clear in the discussion of the alterations in the eye and on the thorax (pp. 110, 111), the duplications seem to be one of the possible results of increased growth in the imaginal buds, and the two last of the three categories above are therefore intimately related. Again, the factors which determine whether increased growth will lead merely to the development of an over-sized organ, or whether duplication will occur, remain for more thorough investigation in the future. The fact, however, that the increase in growth can not only compensate for the destroyed cells, but can result in a considerable excess of material, seems to indicate a greater degree of indeterminacy than might have been expected. The only previous experimental evidence of a capacity for overgrowth in the imaginal buds showed that this may occur in the eye buds of *Bar* flies (see Bodenstein (1939), Steinberg (1941) for recent discussions). That gene, however, causes the development of abnormally small eyes, and an ability of the reduced eyes to grow towards the normal size does not at all imply the ability, which is shown by the present material, for wild-type imaginal buds to grow larger than normal.

Much the most important phenomenon, however, is the first of those mentioned above, the alteration in histogenesis. It has been for long believed that the development of Diptera is highly 'mosaic', that is to say, determined at a very early age. It is clear that from an epigenetic point of view, the embryonic development of the larva and the pupal development of the adult are two different systems; and the greater part of the evidence for the mosaic character of dipteran development admittedly relates to the former, and can only be extrapolated to the latter with some hesitation. But the slight evidence available as to pupal development also seemed to indicate that it was rather rigidly determined, in considerable detail, at an early stage. Thus Geigy (1931) observed the formation of defects in imagos developed from eggs which had been injured by ultra-violet puncture a few hours after fertilization. Howland & Child (1935) obtained similar results with mechanical injuries. Wolsky & Huxley (1936), on the other hand, suggested that the determination of ommatidia in the eye only took place during pupation, but Chevais (1937) pointed out that good development of ommatidia occurs in the eye disks transplanted from late larvae into abnormal situations in the body. More recently, Bodenstein (1939) pushed back the earliest time at which ommatidial development could be obtained to the 50 hr. (after hatching) stage; and Steinberg (1941) has since obtained good development of eye disks aged only 24 hr. after hatching.

Although, on the basis of the experiments just mentioned, Steinberg is impressed with the early determination of the imaginal buds in *Drosophila*, he brings forward evidence that this determination is not in all cases absolute. In *Bar* eyes the number of ommatidia is much reduced, some of the normally ommatidial material being converted in body surface. Steinberg shows that the area which is affected by this conversion can be changed by procedures acting even in the later half of larval life. He therefore supposes that the imaginal bud is determined as a whole, to develop into an eye with the associated part of the head, but that the exact fate of the individual cells is still susceptible of modification. Exactly parallel cases are, of course, well known in amphibian development, for instance, in the eye cups discussed by Spemann (1938, Chap. III). It is clear, however, that this degree of lability does not go nearly far enough to explain the present results. Steinberg contemplated only the possibility of shifting slightly the boundary between tissue which develops into ommatidia and that which becomes body surface. The formation of eye palps would already be a more exaggerated case of this than any he was concerned with. A still more radical reorganization would be necessary for the production of duplications, such as those of the wings. Even they could be regarded as redistributions of potentialities already present within the bud. The greatest degree of modification obtained in these experiments went even further; in the formation of antenna-like palps on the eye, a change of developmental fate outside the range of the eye region has occurred, and the same is probably true of the formation of body surface on the wings.

It appears then to be incontrovertible that very considerable alterations in development are still possible as late as the $3\frac{1}{2}$ day larva. Moreover, as was seen above, the immediate causes of such alterations must be sought in abnormal pervasive conditions (chemical or physical) which can arise within the tissue. That is to say, the cells of the imaginal tissue can themselves fairly easily produce substances or conditions which can spread into the surrounding tissue and alter its developmental fate. Such a phenomenon is obviously similar, in general terms, to the well-known organizer process which plays such a prominent role in the development of vertebrates.

There is no necessary contradiction between this conclusion and the earlier experimental results. Experience with the processes of vertebrate development has abundantly shown that the development of a mass of tissue may remain unaltered under one treatment (whence the material appears 'determined'), while some other treatment may change it (whence the material appears still labile). Determination, in fact, is experimentally always a relative and not an absolute term. We need not be at all surprised to find that, even though an imaginal bud is still labile, its fate is not altered by mere isolation into an abnormal situation.

A few words are necessary about the increased growth which has been described in these irradiated animals. No measurements of growth rate have been made, nor has there been any exact measurement of the sizes of the various parts of different flies. Any attempt to give a quantitative estimate of the increase in growth would meet with very great difficulties, both because of the difficulty in isolating the derivatives of the various imaginal buds, and because of the well-known dependence of the absolute size of the animals on the nutritive conditions. Mere inspection, however, reveals that, in a given individual, the derivatives of a certain imaginal bud may be over-sized in comparison with the rest of the body. It is this relative hypertrophy of particular regions that has been spoken of as an overgrowth. It must certainly be taken to indicate that the over-sized imaginal bud has formed a greater mass of tissue than it would have done if untreated. The alternative explanation would be that the irradiation had tended to cause a general diminution in size, but that certain imaginal buds were less strongly reduced than the majority. But this can be excluded, since the reduction in size of the whole animal is not sufficient to account for the relative hypertrophy of the overgrown parts.

An increase in growth is not a common effect of irradiation by X-rays. In fact, it seems doubtful if any other satisfactory case can be found in the literature. The phenomenon is, of course, not to be confused with the transitory increase in growth rate, which may follow and compensate for the decline in mitotic frequency caused by small doses of radiation (Spear, 1931). In the earlier literature, a few cases of accelerated growth following irradiation have been claimed. Thus Gilman & Baetjer (1904) claim that irradiated *Amblystoma* eggs became larger than the controls during the first few days after radiation; but no data as to the dose employed are given, and since the embryos (presumably irradiated immediately after fertilization?) would be living on stored yolk during this period, the significance of the observation is obscure. Bardeen (1911) has described monsters in radiated amphibian embryos in which there were some outgrowths of the epidermis; but it appears from the descriptions that the animals were always extensively deformed, and the outgrowths were probably due rather to the failure of the normal elongation of the dorsal axis than to any excessive formation of epidermal tissues.

Thus no case at all closely analogous to the present one seems to have been described. Presumably the excessive growth in irradiated *Drosophila* must be attributed to a stimulative effect of some of the substances released by the cells killed by the X-rays. It is perhaps worth pointing out that the greatest growth of the imaginal tissues normally takes place in an environment containing a large amount of necrotic tissue, that is, inside the puparium where active histolysis of larval tissues is proceeding. It may well be that the growth of dipteran imaginal tissues is favoured by such an environment.

No increases in total growth occurred in the animals irradiated at very early stages.

This indicates the activity, in those stages, of a growth-regulating system capable of suppressing the overgrowth found in the animals irradiated at an older stage. The occurrence of such a regulation in growth during the early period is perhaps connected with the regulation of bristle-forming cells which also occurs during the same phase of development.

The absence of abnormalities of histogenesis in the early irradiated animals which show complete growth regulation, strongly suggests that the effects on histogenesis are causally dependent on the growth effects. In the particular case of the conversion of eye tissue to palp tissue, it has already been argued that the histological change is a consequence of the abnormal folding which is a sequel to overgrowth. The general parallelism in the occurrence of abnormal histogenesis and abnormal growth makes it probable that mechanisms of a similar kind may be concerned in the development of other organs, but definite anatomical evidence of this is not yet available.

SUMMARY

1. *Drosophila* larvae of various ages and several genotypes were subjected to heavy doses of X-rays (7000 r. units).

2. (*a*) In pupae aged 27 hr. after puparium formation, no morphological abnormalities were produced.

(*b*) In late larvae (aged more than 90 hr. after laying) and young prepupae, the main effects were: roughening of the eyes, destruction of many macro- and microchaetae and of cells of the wing, arrest of morphogenesis of the wing, and faulty eversion of the legs leading to crippling.

(*c*) In mid-larvae (aged 60–90 hr. after laying) the bristles were usually normal. The main effects were: overgrowth of parts, leading to the formation of over-sized organs and/or excrescences, e.g. on the wings; reduplication of organs, e.g. of wings, eyes, parts of the thorax, legs, etc.; changes in histogenesis, e.g. the formation of palps from presumptive eye material, of leg-like thickenings of the arista, of body-surface material from wing epithelia, etc. The eye palps may have the character of antennae, or extra ocelli may be formed.

(*d*) In younger larvae the effects were less marked, and the youngest groups irradiated gave quite normal adults.

3. The effects on late larvae (groub *b*) can be simply explained as the results of the killing of individual cells, although the unduly frequent destruction of both a bristle and its accompanying socket indicate some interaction of the trichogen and tormogen cells during development.

4. The effects on mid-larvae demonstrate that the determination of the imaginal buds is by no means final at this stage, and a considerably greater degree of plasticity, both in growth and histogenesis, must be assumed than has previously been suggested. Moreover, the fact that the abnormalities affect large masses of tissue indicates that they are caused by agents (chemical substances or physical conditions) capable of spreading throughout the affected region. These agents must be producible by the affected cells themselves. This suggests that determination in normal development occurs fairly late, and through the agency of tissue interactions.

5. Attention is drawn to the occurrence of an increased quantity of growth following irradiation, but no explanation is offered for this phenomenon.

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THE EFFECTS OF SOME OESTROGENIC SUBSTANCES AND CYCLIC HYDROCARBONS ON THE RATE OF GROWTH OF CELLS

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INTRODUCTION

In view of the fact that the oestrogenic substances play a part in the aetiology of mammary cancer in mice it seems important to investigate the effects of these substances on the growth of cells in general, apart from their action on the reproductive organs.

The existing literature is scanty and may be summarized as follows:

Portes, Lantz & Krajevitch (1939) found that oestradiol enhanced the growth of colon bacilli in vitro. Tavastcherna (1936) obtained stimulation of the rate of growth of heart-tissue cultures with follicular hormone. This is supported by Von Haam & Cappel (1939), who report that oestrin in a dilution of 10^{-7} caused slight stimulation of the growth of mouse-heart fibroblasts, as indicated by an increase in the area of the culture and a rise in the mitotic index. Higher concentrations were found to have an inhibitory effect. Maeda (1936), using a preparation called ovaglandol (exact nature unknown), obtained an increased rate of growth of iris epithelium cultured in vitro. Yagi (1937), using rabbit tissues grown in vitro, found ovahormon (nature unknown) to stimulate the growth of uterine, ovarian and testicular tissues, but to inhibit the growth of dental pulp, spleen and bone marrow.

Any comparison of these results is impossible, since dosages, methods of application and the agents used have varied.

The present work was undertaken in order to examine the effects of these substances on a different cell type, namely, a free-swimming Protozoan, and if possible to afford confirmatory evidence to some of the above work.

MATERIAL AND METHODS

The animal used was *Colpidium* sp. It was grown in pure culture in a medium consisting of equal parts of glass-distilled water and Peters's solution containing 15×10^8 *Staphylococcus albus* per c.c. The stock cultures were maintained in small glass pots. Throughout the experiments precautions to ensure sterility of the apparatus and reagents used were taken, as it was found that bacterial infection, with consequent effects on growth rate, occurs very easily.

As the basis of each experiment a healthy and rapidly dividing culture was taken and spun at low speed in the centrifuge for a few minutes to remove any debris. Within a short time the animals had swum to the surface and were then removed with a pipette. From this culture samples were then taken.

The method of sampling was to allow the culture to run up a capillary tube until the level of a mark on the tube was reached. The outside was wiped dry and then the contents delivered into a well slide down to the level of a second mark by holding the tube vertically. By this means it was found possible to achieve a high standard of accuracy of sampling.

For each experiment ten samples were taken from the same stock culture into well slides. All received two drops of the *Staphylococcus* emulsion in Peters's solution. Five, to be used as the controls, received two drops of glass-distilled water each, and the other five, as the experimentals, received two drops of the oestrogen each, the concentration of the oestrogen being twice that required in the final medium.

The slides were placed in pairs in Petri dishes which contained a layer of moistened blotting paper. All cultures were kept in the incubator at a temperature of 20° C. After 24 hr. the animals in both the control and experimental cultures were killed by the addition of one drop of Susa per slide. This caused the animals to sink to the bottom of the slide. Then the total number of animals per slide was counted, a low-power microscope fitted with a moving stage being used for the purpose.

The oestrogens used were oestrone (Organon), oestradiol benzoate (Organon) and stilboestrol (B.D.H.). They were initially made into 1 : 5000 crystalline suspensions in glass-distilled water by dropping an acetone solution into water and then pumping off the acetone. Further dilutions were then made of these suspensions. The oestradiol benzoate and the stilboestrol were found to remain in suspension even at extreme dilution. Oestrone was in solution at a dilution of 10^{-5}. Higher concentrations always contained some crystals.

RESULTS

Attempts to obtain figures using oestrone in dilutions of less than 10^{-5} were unsuccessful. Growth was very erratic, and in many cases the substance proved to be lethal. This was probably due to the fact that crystals were present, and whilst some animals ingested these and received very heavy doses, others only received the effects of that part in solution.

Results for lower concentrations of oestrone, where solution was complete, are given in Table 1. The data have been examined according to the method of Student's 't' test. The probability values obtained are shown in the table. (For details of the method see Fisher (1941).)

All P values quoted refer to both tails of the distribution. In the case of 10^{-7}, since there is an expectation we may use the half value of P, 0·0194, as a test for significance. This indicates that the difference between the two sets of figures is not due to random sampling errors. In the case of 5×10^{-8} there is obviously no significant difference between the sets of figures.

Using oestradiol benzoate and stilboestrol no detailed results could be obtained. In both cases dilutions down to 10^{-7} were found to be lethal. Lower concentrations gave anomalous results. Here again this was probably due to the presence of crystals in the medium.

Results of some tests to see whether the proliferative effect of the oestrone was maintained over periods longer than 24 hr. are shown in Table 2. Cultures were seeded with forty organisms picked from a rapidly growing culture, and at the end of 24 hr. subcultures of the same number of animals were made from each slide. The remainder were then fixed and counted. This was repeated day by day. Stimulation was found to occur for a time and then retardation set in, became progressively more noticeable, and eventually led to the death of the animals.

Table 1. *The effects of various dilutions of oestrone on the total number of animals in a culture over a period of 24 hr.*

1×10^{-5} oestrone				1×10^{-6} oestrone				1×10^{-7} oestrone		5×10^{-8} oestrone	
Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.
1695	2022	1142	1318	595	668	957	1051	2574	3083	1098	1039
1543	1917	1097	1198	568	756	1071	1287	2430	3168	1027	975
1677	2155	1211	1279	478	693	1053	1243	2528	2974	931	1026
1485	1996	1195	1389	493	643	987	1127			1017	968
1607	2126	1168	1336	585	724	967	1095			1034	1059
1601 (*M*)	2043 (*M*)	1163 (*M*)	1304 (*M*)	544 (*M*)	697 (*M*)	1007 (*M*)	1160 (*M*)	2559 (*M*)	2952 (*M*)	1021 (*M*)	1033 (*M*)
$t=7{\cdot}502$ $n=8$ $P=0{\cdot}00007$		$t=3{\cdot}752$ $n=8$ $P=0{\cdot}0056$		$t=5{\cdot}177$ $n=8$ $P=0{\cdot}0008$		$t=3{\cdot}062$ $n=8$ $P=0{\cdot}015$		$t=2{\cdot}637$ $n=6$ $P=0{\cdot}0388$		$t=0{\cdot}362$ $n=8$ $P=0{\cdot}73$	

(*M*) is the mean value of the column above.

Table 2. *Total number of animals in the culture at the end of the period* (*in days*)

Days ...	1	2	3	4	5
Control	679	696	675	660	643
Experimental	871	746	634	194	Dead
Control	614	658	632	625	—
Experimental	788	607	349	Dead	—

DISCUSSION

From the foregoing it is seen that oestrone in dilutions of 10^{-5} to 10^{-7} is effective in stimulating the rate of growth of *Colpidium* sp. when contact with the reagent is not maintained for more than about 24 hr. No conclusions can be drawn as to the action of oestradiol benzoate or stilboestrol in this respect.

From the present work and the existing literature it is apparent that the oestrogenic substances must be regarded as growth stimulators, this growth-stimulating effect being a general one and not restricted to the tissues of the reproductive organs.

The significance of the reversal of the stimulating effect of oestrone when the observations are extended is not clear. It may be that the oestrone is retained in the cytoplasm of the animals and that a toxic and eventually lethal dose is accumulated. This effect of stimulation followed by retardation may account for some of the discrepancies in other workers' results.

Comparison of some effects of oestrogenic compounds and cyclic hydrocarbons

Many authors have drawn attention to chemical similarities between the cyclic hydrocarbons and the oestrogenic substances. Further, there are similarities in biological behaviour and both have been shown to play a part in the aetiology of cancer. Evidence of the oestrogenic properties of the cyclic hydrocarbons has been obtained by Cook & Dodds (1933) and Perry (1938). Waddington & Needham (1935) and Waddington (1938) have found that carcinogenic and oestrogenic substances are some of the most active compounds in causing the induction of a neural tube in Amphibian gastrulae.

Here an attempt is made to compare the effects of the oestrogenic substances and the cyclic hydrocarbons on the rate of growth of the lower organisms and the isolated tissues of the higher animal. With regard to lower organisms, thirteen papers have been found. In three cases (Goldstein, 1937; Hopper & Clapp, 1939; and Spencer & Melroy, 1940) bacteria were used, but graphs and data are given in only one paper (Goldstein, 1937). Increased proliferation with carcinogenic hydrocarbons was in all cases observed, but not with non-carcinogens.

Wright & Anderson (1938) used the mould *F. lini*, exposing it to ultra-violet irradiated dibenzanthracene; they found an initial inhibitory period followed by increased proliferation, as measured by increase in weight and utilization of glucose. In three papers yeast was employed, in one (Hollaender, Cole & Brackett, 1939) neither dosage nor data were given; in the other two papers (Cook, Hart & Joly, 1938, 1939) data were given; at $3{\cdot}6 \times 10^{-3}$ *M* of dibenzanthracene for 24 hr. retarded growth was observed, and with $3{\cdot}6 \times 10^{-4}$ increased rate of proliferation. Two observers (Mottram, 1939 and Wolman, 1939) used *Paramecium*: increased proliferation based upon good data was found with the carcinogens in dilutions of about 1×10^{-6}, but not with four non-carcinogens. Hammett & Reimann (1935) found increased rate of the production of buds and an increased metabolic rate, when methylcholanthrene or dibenzanthracene in saturated solutions was applied to *Obelia geniculata*. Owen, Weiss & Prince (1938) observed an enhancement of the rate of regeneration of new heads in the case of the planarian, *E. dorotocephala*, using saturated solutions of the hydrocarbons, as well as an increase in the number of the regenerated animals. The same workers (Owen *et al.* 1939) record increase of the rate of growth of rootlet transplants of *Pisum sativum*. There is therefore complete unanimity in the records of the action of the carcinogenic hydrocarbons on lower organisms, and increased proliferation of the cells under suitable dosage.

Considering, now, the effects upon tissue cultures of mice, rats and fowls, it will be seen that there is little agreement. In many papers neither data nor statistical analysis was given; they are therefore of little importance. Bisceglie & di Grazia (1936) used 3·4-benzpyrene on chick-heart tissues in dilution of 1 in 500 to 1 in 10,000 and found that growth remained luxurious. Larionow, Ivachentzova & Tchertkova (1938) used 0·02–0·05% and obtained transitory stimulation; in another paper (1941) $1{\cdot}5 \times 10^{-6}$ and 6×10^{-6}, and observed secondary areas in the cultures proliferating more rapidly than the original tissues, this after 1½–6 months' exposure. Lebenzon (1938*a*, 1938*b*) used saturated solutions and obtained retardation in rate of growth, but with small concentrations (1940), not specified, acceleration. Lewis (1935), with concentrations up to 0·1%, found no interference with growth during 24–48 hr.; Magat, Lebenzon & Volkeson (1937) found no effect on either growth or metabolism, but the concentration of dibenzanthracene used was not given. Mauer (1938), in concentration of 1 in 40,000 to 1 in 400,000, found degeneration and death after 4–5 transplants, as did also Timofeevski & Benevolenska (1940) in concentration of 1 in 1000 to 1 in 1,000,000 for 3 days to 3 months. It is doubtful if any sound conclusion can be drawn from these experiments when no measure of effect was made, other than visual impressions. Degeneration and death as compared with controls is perhaps valuable and for the most part was obtained only with high concentrations of the hydrocarbons.

Dealing now with those observers who give data and statistical analysis, four papers are available: Timofeevski & Benevolenska (1939) obtained no evidence of the stimulation of the rate of growth of rat tissues and bone marrow and rabbit leucocytes during 1 month; the concentration used varied from 1×10^{-3} for dibenzanthracene to 1×10^{-6} for methylcholanthrene. Earle & Voegtlin (1938) likewise obtained no stimulation using methylcholanthrene, 2×10^{-3} to 2×10^{-7}, on tissues of rats and mice. They continued for 4 months, and at the end found only degeneration and death of the cells. Creech, in two papers (1939, 1940), used fibroblasts from mouse embryos and applied choleic acid compounds of dibenzanthracene, 1×10^{-5}, and of methylcholanthrene, 1×10^{-6}, for periods up to 70 hr. He obtained increased proliferation as measured by mitotic index and outgrowth measurements; in concentration of ten times the above, retardation was obtained. With the non-carcinogen phenanthrene, 1×10^{-4} to 1×10^{-5}, only retardation occurred.

It must be concluded, therefore, that in the case of tissue cultures increased proliferation, though sometimes found, is by no means observed regularly; however, conditions of dosage varied greatly both in concentration and time of application; for the most part prolonged exposure led to degeneration and death, whilst stimulation was a short-time effect under very low concentrations. In tissue cultures, the cells are suspended in a fluid rich in proteins and lipoids with which the cyclic hydrocarbons are known to combine, and this may well greatly modify the dosage to the cells, whereas in the case of the lower animals, the medium used was usually water or one of simple composition.

Taking into consideration experiments on both the lower organisms and tissue cultures it seems reasonable to draw the conclusion that both the oestrogenic substances and the cyclic hydrocarbons will, for a short time, act as stimulators of growth provided that they are only present in very low concentrations. Higher concentrations are toxic, leading to degeneration and death. Where contact with the chemical is maintained for more than a few days the evidence indicates that there is a divergence between the effects with the lower organisms and with tissue cultures.

SUMMARY

1. Oestrone in dilutions of 10^{-5} to 10^{-7} was found to stimulate the growth of *Colpidium* sp. over periods of about 24 hr.; with longer exposures retardation and death occurred.

2. Higher concentrations of the oestrone were found to be lethal; whilst lower ones had no detectable effect.

3. Owing to the impossibility of obtaining suitable solutions of oestradiol benzoate and stilboestrol anomalous results were obtained.

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THE TEMPERATURE AND HUMIDITY RELATIONS OF THE COCKROACH

VI. OXYGEN CONSUMPTION

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(With One Text-figure)

I. INTRODUCTION

In recent years a considerable amount of work has been done on the rates and the mechanisms of water loss by evaporation in insects (e.g. Uvarov, 1931; Buxton, 1932; Mellanby, 1935; Ludwig, 1936; Ludwig & Landsman, 1937; Thornthwaite, 1940) and on the behaviour reactions of insects in humidity gradients (e.g. Gunn & Kennedy, 1936; Fraenkel & Gunn, 1940; Pielou, 1940; Wigglesworth, 1941). A certain amount of information has been published about effects of air humidity on metabolic rate (Hennings, 1907; Caldwell, 1925; Uvarov, 1931; and others quoted below), but a clear distinction has not been made between direct effects of dry air and indirect effects due to a desiccated condition of the insects.

Caldwell (1925) used an Osterhout apparatus to measure the carbon dioxide production of mealworms (*Tenebrio molitor* L.) and some other animals when starved and desiccated. Since no special method was used to control the humidity in this apparatus, presumably the air was kept moist by the fluids normally present in it. A few batches of three animals were tested over a desiccation period of 6 days, a very short period for the mealworm (Buxton, 1930), and there were undesiccated controls. The controls did not show a constant metabolic rate; in the first 3 or 4 days the variation was seldom greater than 2 or 3 %, but by the sixth day the rate had fallen by 20–30 %. This fall was presumably due to the cessation of digestive processes. A similar fall occurred with desiccated animals. Presumably the appropriate way of allowing for this is to compare the change in rate for desiccated animals with that for undesiccated animals starved for the same time. On the third day the average rate for desiccated animals had risen absolutely and was 26 % higher than that for undesiccated controls. On the sixth day the desiccated animals were producing slightly less carbon dioxide per gram of original (undesiccated) weight than the controls. Caldwell concluded that there was 'a definite, positive, correlation between desiccation and carbon dioxide production'. The only justifiable conclusion, however, is that after 1–4 days' starvation desiccated animals produced carbon dioxide faster, when tested in moist air, than undesiccated controls.

It was tentatively suggested by Buxton (1930) that mealworms desiccated for long periods can make up some of the lost water by the oxidation of larger amounts of reserve foods; the suggestion was based on the dry weights of small numbers of insects after desiccation at 23° C. Mellanby (1932*a*) found, on the basis of chemical analyses, that no extra metabolism occurred in mealworms desiccated at 30° C. In later work he concluded that there was no greater loss of dry weight in dry air than in moist in *Cimex* (1932*b*), *Tineola* (1934), *Glossina* (1936*a*) and *Tenebrio* (1936*b*). Mellanby (1936 *a*, *b*) further pointed out that the beneficial effect of producing extra metabolic water would be offset by an increase in water loss, for the increased metabolism would require increased opening of the spiracles.

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* All the experimental work was done by C. A. C.

This work on food reserves does not distinguish between the direct effect of dry air on respiration and the indirect effect through desiccation. A temporary increase of respiration at the beginning of desiccation such as Caldwell (1925) found might not be large enough to show in determinations of food reserves utilized over a long period. Consequently the results of Caldwell and Mellanby are not necessarily contradictory.

In our work on the cockroach the rates of oxygen consumption of both undesiccated and desiccated animals were found in moist air and in drier air.

II. METHODS

Only adult male specimens of *Blatta orientalis* L. were used. They were kept at 25° C., the temperature at which respiration was measured. They were deprived of food for 48 hr. before each experiment with a view to measuring standard metabolism. Desiccated animals had been kept, unfed, in dry air for 3 days. Some died during that time, but usually they lived and lost about 25 % of their initial weight (cf. Gunn, 1933).

Oxygen consumption was measured in a Barcroft apparatus with a specially designed bottle (Fig. 1 *e*). A solution of caustic potash of appropriate concentration covered the whole floor of the bottle and served both to absorb carbon dioxide and to control the air humidity. The cockroach could move about in a basket made of perforated zinc but could not touch the potash. The potash solutions were made up to be in equilibrium with atmospheres at 10 and 95 % R.H. according to data given by Buxton & Mellanby (1934).

There is considerable individual variation in rate of oxygen consumption of cockroaches, and in one individual, for example, the rate varies from time to time because of activity, feeding and moulting (Gunn, 1933, 1935 *a*, *b*). It was therefore necessary to design and control the experiments carefully. Two Barcrofts were used together, one for drier air and the other for moist, each with a single animal. After a 20 min. equilibration period and observations usually lasting 60–90 min. the animals were interchanged and tested again. Thus each animal was tested at each humidity, with a short interval between the tests. On the next occasion the Barcroft which had previously been used for moist air was used for drier; this tended to compensate for any calibration error. When a particular animal was tested in more than one pair of experiments the order in which it was subjected to moist air and to dry was alternated in successive pairs of experiments; this was to allow for any trend in rate of respiration.

On the whole the cockroaches were remarkably inactive during the experiments. It is well known that variable activity or any variation in rate of oxygen consumption makes the Barcroft an unsuitable instrument because of delay in absorption of carbon dioxide. In these experiments the manometer differences were plotted against time, and only those which gave a straight line were accepted. Experiments in which activity occurred other than one or two changes of position were rejected.

III. CALIBRATION OF THE APPARATUS

The Barcroft respiration apparatus is usually employed for wet tissues or for animals in a saturated atmosphere. For our experiments, in which the air was sometimes moist and sometimes drier, it is necessary to consider whether the results would be falsified by a humidity effect; when oxygen is absorbed the pressure will fall more in saturated air than in dry because some of the water vapour will liquefy. It is not necessary to repeat the complete calculations leading to the calibration constant, for they have been dealt with by Dixon (1934).

Let V cu.mm. be the volume of each bottle, assumed equal,
v cu.mm. be the volume of oxygen consumed in a given time at pressure $(B-w)$,
B mm. Hg be the barometric pressure,
w mm. Hg be the water vapour pressure in both bottles,
Pe mm. Hg be the pressure in the experimental bottle after the given time,
Pc mm. Hg be the pressure in the control bottle after the same time,
d mm. be the difference of levels along the manometer scale (initially zero) after the same time,
k be the factor required to convert d into a pressure difference in mm. Hg,
a sq.mm. be the cross-sectional area of the manometer tubing.

Since all the experiments were carried out at one temperature, temperature may be omitted from the following equations.

For the control bottle the weight of dry air present remains constant, though the weight of water vapour present in bottle and manometer tubing will increase because of the movement of the manometer fluid.

For the dry air $$(B-w)V=(Pc-w)\left(V+\tfrac{1}{2}da\right),$$

$$Pc=w+\frac{(B-w)V}{V+\frac{1}{2}da}. \tag{I}$$

For the experimental bottle the dry air of volume V at pressure $(B-w)$ is reduced by the absorption of air (oxygen) of volume v at pressure $(B-w)$. The weight of air left after the given time therefore originally occupied $(V-v)$ at $(B-w)$; afterwards this expands to fill the bottle, but its space is reduced by the manometer fluid rising:

$$(B-w)(V-v)=(Pe-w)\left(V-\tfrac{1}{2}da\right),$$

$$Pe=w+\frac{(V-v)(B-w)}{V-\frac{1}{2}da}. \tag{II}$$

The difference of pressure is shown by the manometer

$$kd=Pc-Pe. \tag{III}$$

Substituting from (I) and (II) in (III):

$$\frac{kd}{B-w}=\frac{V}{V+\frac{1}{2}da}-\frac{V-v}{V-\frac{1}{2}da}.$$

Substituting the following values:

$$k=0{\cdot}06,\quad V=27{,}500 \text{ cu.mm.},\quad a=0{\cdot}7 \text{ sq.mm.},\quad d=200 \text{ mm.},$$

we get $$v=27{,}430\left(\frac{12}{B-w}+0{\cdot}0054\right).$$

Correcting this to standard pressure

$$v' = \frac{27{,}430}{760}(12 + 0{\cdot}0054(B - w)).$$

With $B = 740$ and w either 0 or 23 mm. Hg

$$\frac{v' \text{ for dry air}}{v' \text{ for moist air}} = 1{\cdot}008.$$

That is to say, a given difference of manometer levels will represent 0·8 % more oxygen absorbed in dry air than in moist. If, therefore, the same amounts of oxygen are used in dry air and in moist the readings will falsely suggest that oxygen is used 0·8 % more rapidly in moist air.

IV. COMPARISON OF TWO TYPES OF BARCROFT APPARATUS

Some doubt has been cast on the suitability of this apparatus for experiments with whole animals. Experiments showed that it gave lower values than a chemical analysis method when used for aquatic insects (Fox, Wingfield & Simmonds, 1937). Earlier experiments were done with Barcrofts which had a small surface of potash for absorption of carbon dioxide (Gunn, 1933, 1935 *a*, *b*), and so a comparison was made between results from that earlier type and the new *basket* type.

The earlier type had the potash in an annulus at the top of the bottle, while the glass floor was covered with a carpet of damp filter paper (Fig. 1 *c*). This carpet was necessary to control the humidity, for in its absence wet faeces deposited during an experiment raised the vapour pressure and spoiled the experiment. The cockroach was prevented from touching the potash by means of a copper wire spiral (Fig. 1 *a*). This apparatus is referred to as the *carpet* type.

The volume of each bottle in the carpet type was 21 c.c., while in two Barcrofts of the basket type it was 27 and 35 c.c. The carpet type could be used only with saturated air, so the basket type had to have weak potash in it, to give a high humidity, in this set of experiments.

Ten experiments were done, in which three normal cockroaches were tested separately, first in one apparatus and then in the other, in conformity with the plan described on p. 125. In the carpet type the average rate of oxygen consumption was 308 ± 9 mm.^{3}/g./hr. (at N.T.P.), and in the basket type 296 ± 3 mm.^{3}/g./hr. (average weight of animals 345 mg.). In the whole series, which included some imperfectly paired experiments, the rates were for the carpet type 331 ± 9 and for the basket type 320 ± 10 mm.^{3}/g./hr. The differences are thus 12 ± 10 and 11 ± 13 mm.^{3}/g./hr., with the higher rate in the carpet type; they are only slightly larger than the calibration error of the apparatus.

It may therefore be stated that the differences between these two types of Barcroft apparatus do not make it improper to compare the results obtained from them. It should be understood that the apparatus used was of the old type, not the newer kind with an external burette as described by Dixon (1934, pp. 6–8). The newer type saves troublesome calibration.

V. DESICCATED ANIMALS

The rates of oxygen consumption of seven cockroaches were measured in basket type Barcrofts in both moist air and dry after loss of about 25 % of their original weight by desiccation. In moist air the average rate was 416 ± 33 and in drier air 358 ± 22 mm.^{3}/g./hr.

Both of these figures are considerably above the values already given for normal animals, the rate for moist air being quite significantly higher. These values were calculated on the basis of the weights of the cockroaches immediately before their respiration was measured; when, however, the rates are recalculated on the basis of the normal weight of the undesiccated animals the results are quite different. In moist air the rate was 323 ± 24 and in dry air 287 ± 11 (Table 1 *a*). That is to say, the rate of oxygen consumption *per animal*, when measured in moist air, does not alter appreciably when the animal is desiccated (cf. Table 1 *b*). The most obvious loss of water from desiccated insects is loss from the blood (Mellanby, 1939), a tissue which probably does not consume oxygen at any great rate, so there is no impropriety in referring the rate of metabolism to the normal rather than to the desiccated weight. The lower rate obtained for dry air is dealt with in the next section.

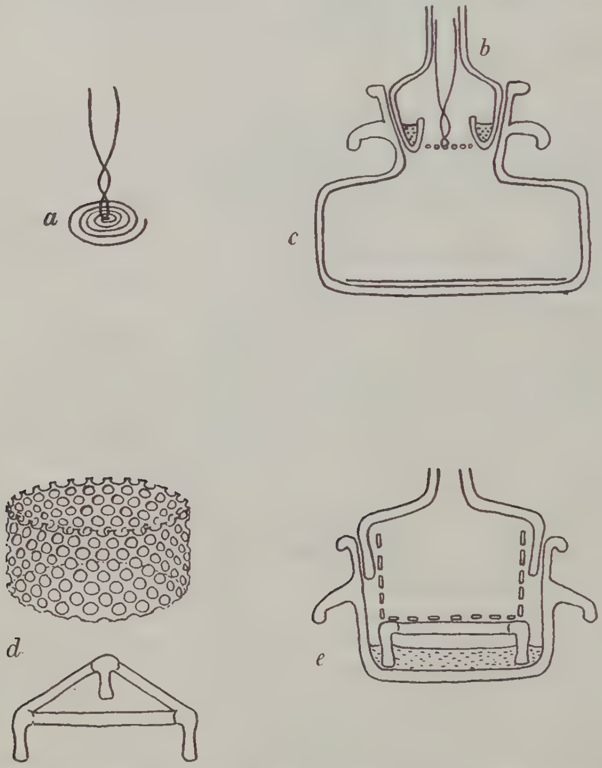

Fig. 1. Details of the two kinds of Barcroft apparatus used. *a*, *b*, *c*, carpet type; *d*, *e*, basket type. *a*, wire coil to prevent the cockroach getting its antennae into the potash; *b*, annular potash container, showing tube for filling and emptying it; *c*, bottle assembled, showing wire coil and potash; *d*, perforated zinc basket and glass stand; *e*, bottle assembled.

There is no evidence, therefore, that desiccation increases the oxygen consumption of a cockroach. A fortiori, there is no evidence that the desiccated condition is relieved by extra production of metabolic water. This agrees with the results of Mellanby, already quoted, for other insects. It is not comparable with Caldwell's (1925) conclusions for the mealworm because his mealworms were hardly desiccated at all.

VI. NORMAL ANIMALS AT TWO HUMIDITIES

The results of properly paired experiments with basket type Barcrofts in which seventeen animals were tested at each humidity are given in Table 1 *b*. It will be seen that rate of oxygen consumption was faster by 36 ± 14 mm.^{3}/g./hr. in moist air than in drier. Making allowance for the humidity error of the calibration (pp. 126–7) this excess is about 12 % of the value for dry air and is 2½ times its standard error. There is a similar difference for desiccated animals (Table 1 *a*) and there seems to be no objection to treating all 24 experiments together. The average excess in moist air is then 36 ± 12 mm.^{3}/g./hr. and, corrected for the calibration effect, the chance probability of this if there is no real difference is less than 1 in 100.

Table 1. *Rates of oxygen consumption of individual cockroaches, each tested both in moist air and in drier air*

	Rates in mm.^{3}/g./hr. Moister air	Rates in mm.^{3}/g./hr. Drier air	Excess rates in moister air
(*a*) Desiccated animals			
	425	255	+170
	405	330	+ 75
	288	243	+ 45
	304	299	+ 5
	272	276	− 4
	297	305	− 8
	268	302	− 34
Totals	2259	2010	+249
Averages	323	287	+ 36 ± 26·3
(*b*) Normal animals			
	341	168	+173
	486	356	+130
	313	216	+ 97
	284	198	+ 86
	300	254	+ 46
	346	302	+ 44
	362	324	+ 38
	357	326	+ 31
	357	328	+ 29
	257	246	+ 11
	209	198	+ 11
	369	371	− 2
	373	377	− 4
	268	282	− 14
	242	256	− 14
	343	359	− 16
	246	285	− 39
Totals	5453	4846	+607
Averages	321	285	+ 36 ± 13·8

Grand totals: moister air, 7712; drier air, 6856; excess in moister air, +856

Grand averages: moister air, 322; drier air, 286; excess rate in moister air, +36 ± 12·1

Examination of Table 1 shows that the distribution about the mean of values of excess oxygen consumption in moist air is markedly asymmetrical. Thus fourteen of the values are below 36 and ten above. Two very high values were derived from experiments in which the rate in moist air was very high or the rate in drier air very low. If these are omitted the average excess is reduced to 23 ± 9·3 mm.^{3}/g./hr. Successive removal of the next two high values reduces the average excess to 18 ± 8·2 and 14 ± 7·6. It is possible that these extreme values are not merely extreme variants in a normal distribution but are due to the intrusion of some large experimental error. Since the average of all the data was 36 ± 12, there is no strong reason for believing the true average to be 36 rather than 24, especially in view of the asymmetry of the distribution. The true average might be as low as 18. All we can infer is its order of magnitude and not its precise value but, in whichever of these ways the data are treated, there is always a larger oxygen consumption in moist air. It is clear from the table, however, that humidity is relatively unimportant amongst the causes of variation in rate of oxygen consumption.

VII. DISCUSSION

The experiments on metabolic rate in two different humidities are of more interest than the others, indicating as they do a higher metabolic rate in moister air both for normal and desiccated animals. There are three kinds of possible reason for the difference found: instrumental reasons, differences of activity and real differences of basal metabolic rate.

The instrumental error due to a humidity effect on the calibration constant has been allowed for. Apart from this a bias would arise if an animal were always tested in moist air before dry air, but that error was carefully avoided. We might regard the error as due to the apparatus if the body temperature of a cockroach were higher in moist air than in dry, for the two sets of experiments would not then have been conducted at the same body temperature and the thermostat would not be adequate. Using the data of Gunn (1933) for the temperature coefficient of oxygen consumption of this species, we find that at about 25° C. a rise of 12% in oxygen consumption is caused by a rise of 1½° C. in temperature.

Now in saturated air, body temperature must be above environmental temperature, for otherwise metabolic heat cannot leave the body; in drier air the body temperature must be somewhat lower. But in these experiments the precise humidities are not known. In moist air there is no reason why the humidity should fall below 95% R.H., and it may be higher because of evaporation from the insect. In drier air evaporation will certainly raise the humidity above 10% R.H., the equilibrium value for the potash; the humidity is unlikely to be uniform throughout the bottle, and no data are available for estimating it. That is to say no precise figure can be given for the difference of body temperature in moist air and drier air in these experiments. Necheles (1924) found differences of 0·4 and 1·6° C. between cockroaches in moist air and in dry at about 25° C., and these differences are of the right order of magnitude to explain the difference in metabolic rate. Koidsumi (1935) found a similar body-temperature difference dependent on humidity in a grasshopper, *Gastrimargus transversus*; he also found a higher metabolic rate, which was of the appropriate size for the body-temperature difference.

A difference of body temperature would alter the calibration constant of the apparatus by altering to some extent the air temperature; but, at the *steady state*, if all the air in the bottle were warmed by 1° C. the error would be only about 0·3% of the rate calculated. On the other hand, if an insect *becomes* active *during* a Barcroft experiment it would warm the air of the bottle and so cause a rise of pressure. A rise of 1° C. would produce, in a bottle of 30 c.c., a pressure increase equivalent to a volume increase of 100 mm.^{3} at constant pressure. This is of the same order as the average oxygen consumption of a blowfly in flight in 2 min. (Davis & Fraenkel, 1940) or of a resting cockroach in 1 hr. It is well known, however, that the Barcroft apparatus gives reliable results only for steady state conditions, owing to lag in absorption of carbon dioxide, and the temperature effect emphasizes this. The heating effect of activity is not a complication in these experiments because experiments with more than the slightest movements were discarded. Apart from the effect of high humidity in raising body temperature and therefore raising metabolic rate the difference found cannot therefore be traced to instrumental errors.

The second possibility is that the animals were more active in moist air than in drier, as they tend to be in some circumstances (Gunn & Cosway, 1938). The full effect of such a difference of activity could not show in these results, however, because experiments were discarded if the activity amounted to more than a few changes of position; but it is still possible that there was sufficient difference in activity to require more oxygen in moist air.

Since slight differences of body temperature and of activity could explain the higher rate of metabolism in moister air there is no reason to postulate a higher basal metabolism in moister air.

Results such as those given above could have been interpreted in various ways if less care had been taken in designing the experiments. For example, desiccation increases the rate of oxygen consumption per gram, though not per animal, while dry air decreases it. That is to say, if no distinction were made between the effect of dry air during the respiration experiment and the effect of a desiccated condition, then it might have been said either that dry air accelerates metabolism or that it retards it. Divergent conclusions in the literature are likely to be due to a failure to make this distinction.

Ludwig (1937) and Bellucci (1939) found no reliable effect of desiccation on metabolic rate in *Chortophaga viridifasciata* De Geer and larvae of *Popillia japonica* Newman respectively, so long as the rates per animal, not per gram, were used. The analyses of the food reserves of various insects carried out by Mellanby (quoted on p. 125), after prolonged subjection to various humidities, do not show any effect of humidity and desiccation on metabolic rate. They are not comparable with experiments in which rates of oxygen consumption are measured during short subjection to various humidities.

VIII. SUMMARY

1. The carpet type of Barcroft respiration apparatus previously used by Gunn for cockroaches gives results comparable with those now obtained with a new basket type.

2. Desiccated cockroaches use oxygen at the same rate per animal as undesiccated specimens. If, however, the rates are calculated with reference to the weight of the animal at the time of the experiment, since the desiccated animals tested had lost about 25% of their original weight, their rates of oxygen consumption appeared to have gone up.

3. Both normal and desiccated animals used oxygen slightly faster in moist air than in dry. Part of this increase must be attributed to a higher body temperature in moist air at 25° C. than in drier air at 25° C. Part of it may be due to greater activity in moist air than in dry, slight though the activity was in both cases.

4. There is no reason to believe that, at a given body temperature, air humidity influences basal metabolic rate.

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THE BIOLOGY AND BEHAVIOUR OF *PTINUS TECTUS* BOIE. (COLEOPTERA, PTINIDAE), A PEST OF STORED PRODUCTS

IV. TEMPERATURE PREFERENCE

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(With Two Text-figures)

INTRODUCTION

Previous papers in this series (Bentley, Gunn & Ewer, 1941; Gunn & Hopf, 1942; Ewer & Ewer, 1942) have provided information about the relations between *Ptinus tectus* and environmental factors, particularly temperature; this paper is concerned with the temperature region in which this species tends to aggregate when placed in a temperature gradient. Determination of this preferred temperature presents certain difficulties which have been recorded by Deal (1941) in connexion with several species of beetles but, as will be seen, on the whole the results fall into place with information already obtained here about *P. tectus*.

APPARATUS AND METHODS

The beetles used in these experiments were descendants of a stock supplied a year earlier by the Biological Field Station of the Imperial College at Slough. They were bred in 2½ l. jars in 300 g. of the usual wholemeal flour and yeast mixture. The animals were always allowed to drink before an experiment.

The temperature gradient apparatus mainly used was the one described by Gunn (1934, 1935), and a second kind of gradient apparatus is dealt with on p. 137. The former has been silver-plated, for use with aquatic organisms, and the cooling was done by putting ice or solid carbon dioxide in a box fixed to one end, instead of by circulating cold alcohol around that end. By means of solid carbon dioxide a temperature of −6° C. could be maintained at the cold end and the insects could not reach the end corners; there was no difficulty about keeping the hot end of the gradient hot enough to keep the insects away. Interpretation of the results was therefore not complicated because of reactions to the ends of the apparatus. In order to eliminate the tendency of the insects to collect round the thermometers (Deal, 1941), the holes for them were in the double glass roof, instead of in the side walls. Instead of the insects being allowed to walk on the metal floor, when the smallest irregularities lead to aggregation in response to the extra contact stimulation, a false floor of fine bolting silk was slung from the sides of the apparatus, providing a trough-shaped run for the animals. In order to remove smells left by the animals, which seem to be an important circumstance causing aggregation in places where aggregation has occurred before, the bolting silk was washed and ironed after each experiment. This material was more satisfactory than smooth metal, for when the beetles tumbled over on to their backs they more quickly regained their feet on it than they did on metal.

The apparatus was covered with a black light-tight plywood cover and a curtain which could be lifted for inspecting the animals in the gradient. Inside the cover there were two alternative sets of lamps, one (neon) giving an intensity of about 1 mc. and the other of about 10 mc. around the animals.

The humidity of the air current passed along the gradient could be controlled and measured as previously described (Gunn, 1934). Using the standard range of temperature (−4 to +38° C.), moist air passed in at the cold end would deposit moisture in the entry pipe and so leave the air nearly dry, while if put in at the warm end it would condense in the gradient on reaching its dew-point. If, therefore, condensation in the gradient were to be avoided, the behaviour of the animals could not be tested in moist air, and the only comparison possible was between dry air and nearly dry air. On the other hand, it was possible to investigate the effect of a somewhat higher humidity without much interference with behaviour due to liquid water, with air entering at the warm end, because the condensation appears to have taken place mostly on the metal of the apparatus, while the animals themselves were on bolting silk and could not touch the metal.

It has been shown by Bentley *et al.* (1941) that the frequency of locomotory activity of *P. tectus* varies with a diurnal rhythm, under suitable conditions, and that this rhythm can be controlled by controlling the times of day during which the animals are in the light. In the temperature preference experiments three groups of animals were used: *rhythmless* animals, which had been kept at 25° C. in unvarying light long enough to abolish the rhythm; *day-phase* animals, which were similarly kept, but in artificial light of about 25 mc. in the day (06.00–18.00 hr.) and in darkness at night; and *night-phase* animals, which were kept in darkness during the daytime and in the artificial light at night (18.00–06.00 hr.). Since all the experiments were done in the daytime, day-phase animals were in a rather inactive condition, rhythmless animals were more active, and night-phase animals, with their normal rhythm reversed, were the most active.

The effects of the three variables—light intensity in the experiment, air humidity, and phase of activity—were studied in the twelve possible combinations. Two such sets of experiments were carried out.

The twenty animals used in each experiment were put into the gradient through a thermometer hole at 26–27° C.; this point was chosen because it was not the preferred temperature and not a damaging temperature, but a temperature at which the animals were active. If, instead, animals were initially scattered along the gradient, those put into the colder parts tended to stay there, and records of their positions were not representative of their locomotory behaviour. The positions of all the animals were recorded every 10 min., usually for 5 hr., thus providing 600 observations in each experiment. The direction of the air current was reversed hourly.

RESULTS

The results of all twenty-four experiments added together, including some 14,800 individual readings, are shown in Fig. 1. In Fig. 2 are shown the results of six of these experiments, selected to illustrate particular points.

The average preferred temperature from the data shown in Fig. 1 is 12·1° C. This average temperature is obviously not representative of the behaviour of the species, for fewer animals were recorded at about that temperature than at any other temperature between 1 and 29° C., while considerable numbers were recorded around 4 and 24° C. Fahmy (1931) does not mention the aggregation of *Ptinus* in the cold region; in a few rough experiments, about 40% of the animals settled at 22–25° C., but nothing was said of the rest. Deal (1939) found only a minor aggregation at 20–25° C., while most of the animals collected at the coldest end of the gradient at about 8° C.; the animals were active and not immobilized by the cold and Deal concluded that this aggregation was due to a true temperature preference. He also found a bi-modal distribution of the earwig, *Forficula auricularia*, with a large peak at the cold end and a smaller one at 25–30° C. The bi-modal distribution of *Ptinus* requires further consideration.

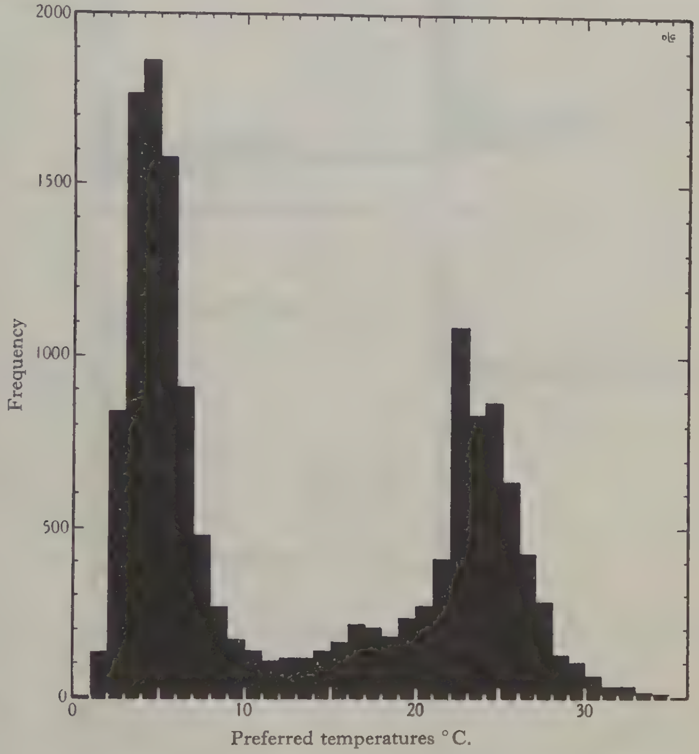

Fig. 1. Distribution of *Ptinus tectus* in the long-temperature gradient apparatus, from twenty-four experiments.

In some of the twenty-four experiments there was little or no aggregation at the high temperature (Figs. 2 *f*, *d*) and in others little at the low (Fig. 2 *b*). Thorough treatment of the data failed to reveal any correlation between the occurrence or absence of these two aggregations and the three variables investigated. Thus Figs. 2 *a*, *b* (also Figs. 2 *d*, *e*) are for the same conditions but do not agree, while Figs. 2 *a*, *e* are for very different conditions but are similar. It therefore appears that the relative magnitudes of the two peaks depended on chance.

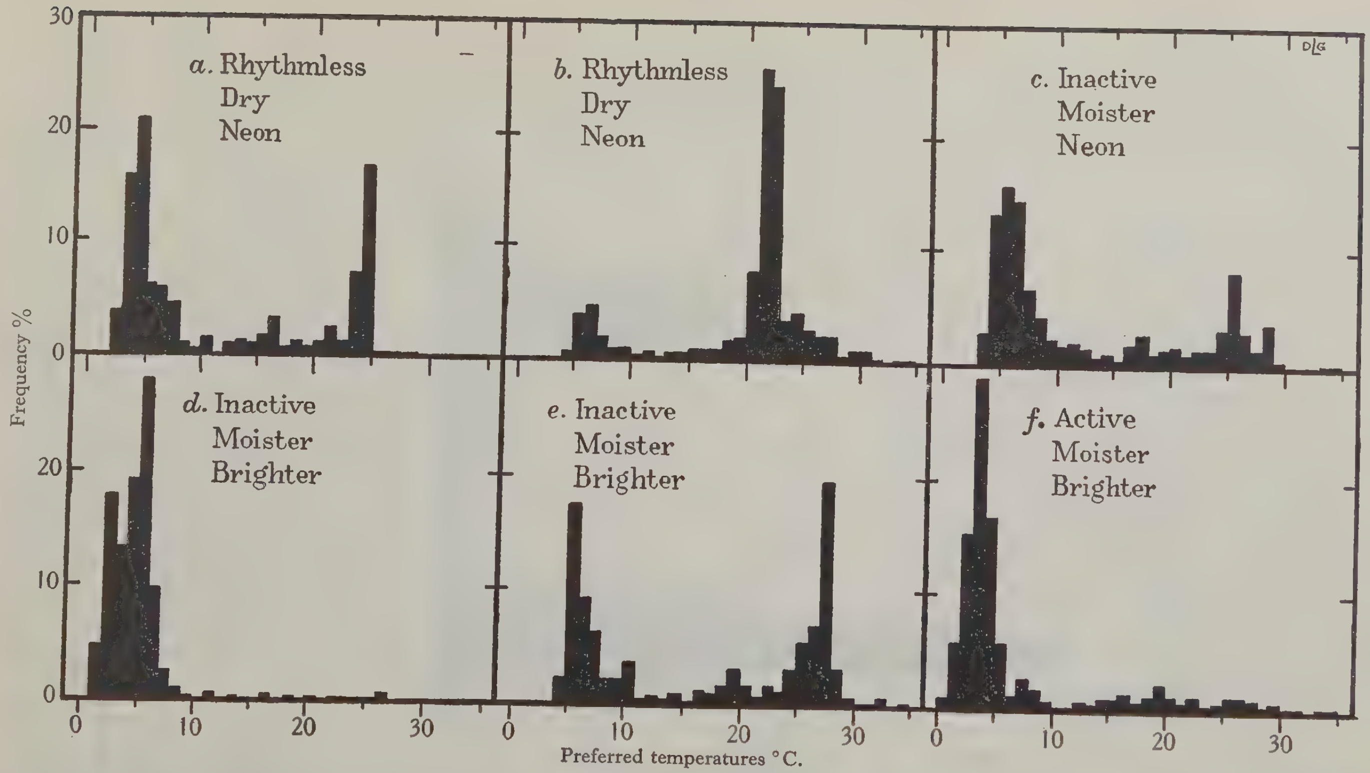

Fig. 2. Distribution of *Ptinus* in six selected experiments in the long-temperature gradient apparatus. Phase of the diurnal activity rhythm, air humidity and illumination as indicated. *a*, an average result (cf. Fig. 1); *b*, few animals at low temperatures, the warm aggregation at a relatively low temperature; *c*, the cold aggregation at rather a high temperature; *d*, no warm aggregation; *e*, the warm aggregation at a high temperature (cf. *b*); *f*, the only case in which animals penetrated below 1° C., cold aggregation at a low temperature (cf. *c*).

In view of this conclusion it is worth noticing that in Figs. 2 *b*, *e* the aggregation between 20 and 30° C. does not occur over quite the same temperature zone; in Fig. 2 *b* the peak is at 21–23° C., while in Fig. 2 *e* it is at 27–28° C. These are selected extreme cases, but clearly a single experiment is not enough to show the precise position of such a peak. This is especially true when testing insects which tend to collect in clumps, for the exact position of the peak depends both on temperature and on the place at which a small clump happens to form in the beginning.

In both of the main aggregation regions the animals become immobilized, but apparently for different reasons. At 24° C. *Ptinus* can be active quite frequently and it can walk quite quickly; at 4° C. activity is rare (Gunn & Hopf, 1942), walking is very slow, and immobility is forced upon it by the physiological effects of cold. It seemed rather improbable that an insect would have such poor protection against immobilization by cold as this behaviour suggests, and it seemed likely that the failure of behaviour was due to the form of presentation of the stimulus.

Two features of the apparatus seemed likely to be involved, namely, its shape and the steepness of the temperature gradient. The metal apparatus is long and narrow (122 × 4 cm., Gunn, 1934), and the bolting silk was slung so as to form, in cross-section, the shape of a wide U with the ends turned over to the horizontal. The animals usually walked along the sides of the apparatus with their backs nearly touching the roof. As long as they kept to this line, manœuvring was limited; but this was certainly not the cause of the low-temperature aggregation, for when the bolting silk was stretched horizontally on a frame, such aggregation still occurred. The limitation on manœuvre in the latter case was not severe, for *Ptinus* is only 5 mm. long, including antennae, and it had a width of 4 cm. in which to turn round. The possibility nevertheless remains that the shape of the apparatus canalized the movements of the insects. Some experiments were therefore carried out with a different form of apparatus.

Alternative chamber

The other form of apparatus used was of the alternative chamber type constructed by Thomson (1938). It consisted essentially of a circular glass dish (29·5 cm. diameter and 4·5 cm. deep) with a double glass roof, through which thermocouples were inserted. This chamber was almost submerged in a water tank which was divided into two by a vertical wooden partition. Half of the chamber was on one side of the partition and the other half on the other; the water on the two sides was kept at two different constant temperatures.

The air in the chamber was kept dry with sulphuric acid, and there was a perforated zinc false floor as in the humidity alternative chamber (Gunn & Kennedy, 1936). The temperature did not change sharply over the partition, presumably because of conduction in the zinc, but the isotherms were not sufficiently evenly spaced to allow the temperature at any point to be inferred from a row of seven stationary thermocouples across the chamber; the isotherms could be obtained by rotating the roof carrying the thermocouples into various positions. There were considerable gradients of temperature between the zinc platform and the roof 2·5 cm. above; at about 9 and 29° C. the differences between the temperatures of zinc and roof were about 3° C. at a room temperature of 19° C. This result does not agree with Thomson's (1938) statement that 'there was no appreciable vertical gradient of temperature such as necessarily exists in the usual type of apparatus'. In the long apparatus, the vertical differences of temperature are usually less than 0·1° C., though in Herter's apparatus they may reach 10° C. or more in a height of 10 cm. The situation in the alternative chamber is therefore more complicated than in the long apparatus because of convection currents and because of uncertainty about the precise temperatures to which the animals are reacting; but the alternative chamber has the great advantage that it offers a large arena for manœuvre and it has no corners or ends, in which insects tend to collect.

Results. In this apparatus, with a gradient from 6 to 13° C., there were repeated turns away from the cold part of the chamber at about 10° C., and not one of eight animals tested entered the colder half of the chamber. In a gradient from 8 to 36° C., however, behaviour was much as in the long apparatus, with prolonged periods of inactivity in the cold regions. Thus *Ptinus* can show reactions against low temperatures if the steepness of gradient is suitable. The surprising fact is that it is not a steep gradient but a gentle one which elicits the successful avoiding reactions.

The successful avoiding reactions observed in the gentle gradient were generally of a well-defined type: the animal slows down, or stops altogether, swings the antennae from side to side, then suddenly turns right round and walks off in the opposite direction, the whole reaction taking about 2 sec.

Turning reactions in the steep gradient were not usually of this type. A preliminary analysis of tracks recorded in this gradient showed that turns amounted to about 250, 900 and 1600°/min. at 10, 20 and 30° C. respectively, varying irregularly but without any indication of particularly high values at any special temperature. Average speeds were roughly 6, 22 and 38 cm./min. When the amount of turning was referred to distance travelled instead of to time, it was found to vary around 40°/cm. without any consistent trend, the values for all temperatures lying between 20 and 60°/cm. These figures are based on a small number of observations, but the analysis could not be carried further because the work had to be broken off.

Evacuation temperature

Further evidence was obtained on the difference in behaviour towards high and low temperatures. A score or so specimens were induced to clump near one end of the long gradient apparatus by utilizing their photonegative behaviour. This part of the apparatus was then heated or cooled and a record was made of the temperatures and the movements of the insects. Starting at 25° C. the temperature was raised at about 1° C. in 5 min.; the animals began to leave at 30° C. and all had left for cooler parts at 33° C. When the temperature was lowered from 20 or 25° C. at the same rate or faster, the insects were activated, the clumps broke up and reformed, but less than one-third of the animals left before cold immobilization occurred. That is to say, the upper evacuation temperature is well defined at 30–33° C., but there does not seem to be a lower evacuation temperature at all.

DISCUSSION

It seems likely that behaviour evolves, as structure does, in co-ordinated fashion. If, then, it appeared that the behaviour of *Ptinus* is poorly co-ordinated with its physiology, that would be a matter worthy of closer investigation. A temperature of 24° C., around which *Ptinus* aggregates, is quite favourable for development and egg laying (Ewer &

Ewer, 1942) and for activity (Gunn & Hopf, 1942). On the other hand, 4° C. is probably below the developmental minimum for the species (Ewer & Ewer, 1942) and activity is reduced to a very low level (Gunn & Hopf, 1942). Even a temperature of 8° C., the lowest available in Deal's apparatus (1939) and an aggregation temperature in his experiments, is at least very near the developmental minimum and may be below it. Only eighteen eggs were laid by 200 adults in 6 days at 7° C. (Ewer & Ewer, 1942). If *Ptinus* were frequently to choose such a low temperature in nature the species could hardly survive. The results described above indicate, however, that the occurrence of aggregation at low temperatures depends upon the form of the apparatus used, and if the gradient of temperature is gentle enough the low temperatures are avoided.

It has been shown by Ullyott (1936) that when the flatworm, *Dendrocoelum*, reacts klino-kinetically in a gradient of light, aggregation in the dark region fails to occur if the gradient is not steep enough; sensory adaptation then takes place sufficiently quickly, as the animal moves into brighter light, to prevent effective stimulation of the receptors (Fraenkel & Gunn, 1940). A steep gradient should produce the most effective reaction, culminating in the familiar avoiding reaction where the gradient is steep enough to constitute a boundary. With *Ptinus* in a temperature gradient, however, it is the steep gradient which fails to produce a reaction against cold and the gentle gradient which succeeds. This fact is inconsistent with both the older conception of shock reaction and the new theory of klino-kinesis. Analysis of tracks in the alternative chamber (8–36° C.) provided no support for the latter, but, of course, the animals did not then react against cold. When they did react (6–13° C.) the turnings were not random but resulted in a complete reversal of direction. Unfortunately, experiments with gentle gradients in the long apparatus were unfruitful because of the almost immediate aggregation of the animals at one end or the other.

The steepness of the gradient, besides affecting the state of sensory adaptation, is also important in relation to the lag in body temperature and the consequent lag in adjustment of speed of walking. Little can be said about the lag in body temperature as the animal walks along a gradient. An attempt was made to determine the lag, using a thermocouple, but the only ammeter available was rather sluggish, and the conclusion reached was that body temperature follows environmental temperature over a change of 10° C. with at most a few seconds delay. From the speeds of walking given above, it will be seen that *Ptinus* would normally traverse a gradient 55 cm. long extending from 10 to 30° C. in about 2½ min. That is to say, body temperature is not likely to differ much from environmental temperature as the animal walks about. It is hardly likely that the animal rushes into the cold, with its body still warm, but cannot get away before cooling slows it down too much. In any case, this does not correspond with observation; the animal walks more and more slowly as it approaches the cold end. At the same time it is clear enough that temperature lag would be less in a gentle gradient than a steep one.

The metabolic effect, as distinct from the behaviour one, is clearly important. If an animal overshoots the high temperature at which turning back normally occurs, locomotion is progressively accelerated and a successful reaction at a still higher temperature can and does occur. On the other hand, overshooting the low-temperature reaction point leads to immobilization, and it is rarely that an animal gets away again to a higher temperature. That is to say, a single failure to react against cold leads to immobilization;

a high proportion of the more active animals, which approach the colder regions several times, will be trapped by cold sooner or later.

Low-temperature trapping of insects has been recorded before by a number of authors (see Fraenkel & Gunn, 1940). Because of this phenomenon, Nieschulz (1934, 1935) calculated a 'purified' average preferred temperature which ignored small percentages of flies (*Musca domestica*, *Stomoxys calcitrans* and *Fannia canicularis*) spread over low temperatures. This procedure has the disadvantage of depending on an arbitrary choice of what shall be regarded as a temperature low enough to cause metabolic trapping. Whether an aggregation of *Ptinus* at 4° C. is said to be due to a temperature preference or not is a matter of definition, but it seems more useful to regard only the aggregation around 24° C. as due to the true preference. Errors due to the occurrence of metabolic trapping can be avoided by using suitable apparatus.

SUMMARY

1. In a long temperature gradient apparatus, *Ptinus tectus* aggregates around two distinct temperatures, 4 and 24° C.

2. In a circular temperature alternative chamber there is no marked avoidance of low temperatures if the gradient is steep, but characteristic avoiding reactions occur if the gradient is not very steep.

3. When the stationary animals are subjected to rising or falling temperatures in the long gradient, there is a well-marked *evacuation temperature* at 30–33° C., but none at lower temperatures.

4. A temperature preference around 24° C. is correlated with favourable temperature for growth and other activities. The aggregation around 4° C. is the result of a failure of co-ordinated behaviour in a particular kind of gradient, and *P. tectus* is practically immobilized by cold in that temperature region.

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THE ALTERNATION PROBLEM IN ANIMAL PSYCHOLOGY

EXPERIMENTS WITH FOWL

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The original aim of this paper was to give a contribution to the study of number conception in animals. It has been repeatedly maintained that birds can be trained to take every second and even every third from a row of identical grains. These experiments were carried out with rows of equidistant grains. Thus the bird's achievement was possibly only a training to a certain distance. This eventuality has not previously been properly excluded, for we shall see that the apparently successful results after doubling or halving the usual distances between the grains are not convincing. Neither have attempts been made previously to train birds to select every second or third from a line of unequally spaced grains. Such an achievement, independent of any pecking rhythm and without the 'figural help' of any pattern in the distribution of the grains, could be considered as a preliminary stage to counting. It was therefore intended to train birds first to select every second (or third) from a line of equidistant grains, and to extend this training later to rows with unequally spaced grains. Finally, the row of grains had to be covered by an opaque moving lid, so that it became visible to the bird not as a whole, but gradually. By modifying the speed of the lid any training to a temporal rhythm could be avoided, and by using a lid with a 'window' showing only one or two grains at a time a training to a figural pattern could also be excluded.

In describing the experimental procedure I have attempted to give an accurate account of the bird's behaviour. On the other hand, I have not hesitated to describe the reactions of the birds occasionally in a 'subjective' way—that they appeared to be 'disappointed' or 'discouraged', etc. I wish to state that I am fully aware of the fact that neither disappointment nor discouragement can be proved, and these expressions are used only because they give the shortest possible description of the facts observed and are not intended to indicate more than that.

METHOD AND MATERIAL

The method employed in the present paper was as follows: the bird entered a shifting cage of 60 × 30 cm. (40 cm. high) consisting of wooden struts and a wooden bottom. Four sides were made of wire netting. One small side consisted of a sliding door (plywood, sliding sideways). The actual experiments were carried out in a 'run', 180 × 40 cm. (55 cm. high) also constructed of wooden laths supporting wire netting, which was open on both ends and at the bottom. In this run teak boards were placed to which the grains were glued with seccotine for the training experiments. These boards measured 150 × 15 cm. (2·2 cm. thickness) weighing about 6 lb. each. They were frequently changed as a safeguard against 'secondary cues'. The lid covering the grains was moved by a string coiled round a vertical metal rod at the far end of the run and actuated by an electric motor. A switch in front of the teak board, but outside the run, controlled the movement of the lid. The whole apparatus was placed on a large concrete bench which formed the bottom of the run. The bench stood on a heavily reinforced concrete floor so that any movement of the bench was practically excluded, especially as there was no traffic nearby. Light entered by vertical windows and from skylights.

During the first experiments, shifting cages were used at both ends of the run to prevent the birds from running back after they had pecked grains from the teak board in one direction, but later the far end (to the right of the observer) of the run was closed with a sheet of plywood and only the left or near entrance used.

The food used was barley grains prepared for human consumption ('pot-barley'). These grains were chosen as I knew from previous experience that in the case of rice and shelled maize the grain is slightly transparent, and therefore a glued grain often looks somewhat different from a normal (loose) one. These pot-barley grains have an average size of 5 × 4 mm. with a shallow groove.

The ten subjects used were eight *Gallus domesticus* (mainly Rhode Island Reds) aged 4–6 months at the beginning of the experiments, and a pair of jungle fowls (*G. bankiva*) consisting of a middle-aged hen and a cock, the long spurs of which showed his advanced age. The birds were marked with coloured celluloid rings round their legs. Altogether, more than 4000 experiments were carried out with them.

After some preliminary training in order to accustom the birds to the unusual conditions of eating in the run and returning to one of the shifting cages, the experiments were begun with one cock and three hens.

During the first experiments in order to prevent the grains being moved by scratching, boards with small grooves for each grain were used (Honigmann, 1921), but this was found to be unnecessary and ordinary smooth (brown-stained) teak boards were used. The standard training board had a row of seven fixed grains, 6 cm. apart, alternating with seven loose grains. The normal row consisted therefore of fourteen grains of which the odd ones were fixed and the even ones were loose, with a distance of 3 cm. between every grain and its neighbour.

In detail the procedure was as follows: The bird was enticed to enter one of the shifting cages by means of food and then in the same way transferred to cage 1. The door of cage 1 was now closed and the run tilted about 50°—a wooden bar preventing it from tilting completely (90°). Then the teak board with the fixed grains became accessible to the experimenter as the run had no bottom. The eaten grains were now replaced by new ones and the run returned to its normal stable position—thus covering the board again. Now about 30–40 grains of rice were thrown into cage 1 through the wire netting, and while the bird was eating these grains the sliding door of cage 1 was slowly opened, thus giving access to the run. This feeding with rice took place between all experiments and prevented the bird from rushing into the run as soon as the sliding door was opened, thus allowing the experimenter to go back to a place in front of the run before the bird entered it and started eating barley from the board. The fact that the birds received about 30–40 grains before every experiment did not influence their willingness to eat a maximum of seven grains during the real tests. Rice was chosen as most of the birds like it and as it prevented the birds from getting tired of the kind of grain (barley) used for the actual tests.

After the bird had finished pecking (or eating) it went back to cage 1. The sliding door was now closed and the result of the experiment recorded. Then the next experiment was prepared by replacing the eaten grains, etc.

Factors which disturbed the fowls and prevented them from working satisfactorily included sparrows intruding into the room through open skylights. They were not influenced by pigeons, parrakeets and monkeys in cages in the same room—only about 10 yards away from them. The first snow much upset one hen. Another source of trouble was the sight of food outside the run, so the grain used for refilling the row after the experiments had to be kept in covered boxes.

RESULTS

The first tests carried out with domestic fowls (cock 4, hens 1–3) revealed the expected individual differences in learning. The majority of the birds quickly realized that some grains were not available, but one hen (no. 3) continued for weeks to peck at every grain in the row—loose and fixed. This bird, during preliminary training on rows where all the grains were loose, had proved to be a very reliable worker by eating all grains untiringly, but this reliability was now shown to be a lack of adaptability.

Cock 4 and hen 2 made the quickest progress. As early as the second or third day in several tests, they pecked all the even grains (2, 4, 6, 8, etc.) without touching the odd ones. I had the impression that they made use of secondary cues instead of a true alternation. The glue underneath the fixed grains was hardly visible, but the fixed grains themselves were slightly dusty and therefore darker than the loose ones. Possibly these grains also had some marks visible to the bird. To eliminate both possibilities I darkened the loose grains artificially with dust and repeated the tests. The result was that the birds became confused and made many mistakes. This proved that there were no 'positive' marks on the fixed grains and that the glue did not play any part in the discrimination, but that the birds had learned to eat the grains of a lighter shade and to avoid the darker ones. Numerous tests carried out alternately with normal and darkened loose grains gave the same result.

The fact that in the experiments described by Katz & Révész (1909) and Révész (1922) the birds learnt so very quickly—after only 15–30 trials*—to peck every second grain, or even every third one from a series of grains, was so striking that it seemed important to find out the reason for this marked discrepancy between our results. Accordingly, I repeated the experiments using the technique described in their papers, and used as subject a bird (hen 3) which even after 170 tests had hardly showed any distinct progress. The grains were now glued to pieces of cardboard (15 × 28 cm.) instead of to the heavy teak boards. It was interesting to note that even this hen was able to solve the various 'problems' very quickly. I repeated most of the different tests mentioned in these papers with the same result. But as soon as I made a crucial test in which all the grains on the cardboard were loose the bird ate them all. Pecking on cardboard causes minute movements of the loose grains, and these movements are perceived by the birds. In addition, the appearance of rice grains is somewhat altered if they are glued on to a board or cardboard. But even when barley grains glued on cardboard were used, the effect was similar.

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* Révész (1922, p. 469) repeats the dates given 13 years earlier, but on the following page he states that 40 trials were necessary.

These results make it probable that the apparently successful training to alternate reported in earlier papers was due to secondary cues. On the other hand, there was no proof that such an achievement is impossible. I therefore continued the training, and actually several birds learned to perform a true alternation, i.e. to select every second grain out of a line in which all grains were loose and without any difference in shade, etc. To achieve this, however, not 15–30, but 500–600 experiments performed in 17–20 training days were necessary. It is significant that these figures differed very little in the case of the single birds. We have to consider now each bird separately in order to follow adequately the progress made in each case.

Experiments with cock 4

This bird as we have seen 'trained himself' to eat light grains and to avoid darker ones. This secondary cue was eliminated by darkening the loose grains, but for the following experiments boards were used which were always freshly stocked with barley grains. The last grain in a row proved to have a very strong attraction for the bird and was often pecked afterwards, e.g. 2, 4, 6, 8, 10, 12, 14, 13.

On the 16th day of training the cock selected the grains in perfect alternation during Exps. 1, 3, 5, 12 and 15 out of 25 made during the day, and the following day in Exps. 1, 2, 5, 6, 7. In the next experiment the whole row consisted of loose grains and the result was Exp. 8/493*—eaten 2, 4, 6, 8, 10, 12, 14, 13—the first real success. The next two experiments—again all grains loose—failed, the cock being rather excited, but in Exp. 11/496 he ate 1, 3, 5, 7, 9, 11, 13 (odd ones this time), and in Exp. 12/497 performed in exactly the same way as in Exp. 8/493. Corresponding results were obtained on the following day, e.g. Exp. 10/509 = 2, 4, 6, 8, 10, 12, 14 and Exp. 11/510 = 1, 3, 5, 7, 9, 11, 13, 14, 12 eaten, thus showing that there is no distinct tendency to start with the first grain of the row.

While the previous experiments were carried out with a row of grains having a standard distance of 3 cm. in the following series the first tests were made with unequally spaced grains (Exp. 13/511). The distance between grain 1 and 2 was still 3 cm., between 2–3, 3–4, 6–7 and 7–8, 4·5 cm. and between 4–5 and 5–6, 6 cm. Grains 8–14 had the usual distance of 3 cm. The result was that the cock ate the whole row except four grains (1, 6, 9, 14).

I should now have tried again to offer the same scheme to the cock—a row of fourteen loose grains with the usual distance of 3 cm. each—and perhaps the result would now have been better (see below, p. 146), but I had the impression that some fresh training was necessary and unfortunately used a new board (with fourteen grains—the odd ones fixed) painted black, instead of being stained brown. The bird was completely upset by this unexpected colour, gazed intently at the board and finally started pecking at it, but not at the grains. Even when one of the usual boards was now used he refused to eat, and after three more unsuccessful tests the experiments had to be abandoned for the day.

Next morning the unfortunate impression created by the black board had disappeared and the cock ate quite correctly from a normal board (with the odd grains fixed) all even grains three times in succession; but afterwards he could not refrain from pecking the fixed no. 13. In the next experiment he pecked 2, 3, 4 but otherwise ate all even grains without pecking at odd ones. I mention these details in order to show how impossible it is to predict the behaviour of any bird in a single experiment. During the 6th experiment of the day he had the unusual misfortune to start with fixed grain no. 3 instead of 2, and since his alternating training was retained quite well, he pecked 3, 5, 7, 9, 11, 13, without any success as all these grains were fixed. He became very excited and received plenty of mixed food in order to 'restore his confidence'. He had indeed deserved this 'consolation' as, at this stage of the training, the perfect eating of all odd grains was a positive achievement. In the following test he started correctly with 2, 4, 6 but missed no. 8 and went on to the fixed grains 9, 11—then he stopped again 'disappointed'. The next two experiments were still very unsatisfactory but 10/538 gave a perfect result. I therefore took the risk of offering again a test row (all fourteen grains loose) and he ate correctly 2, 4, 6, 8, 10, 12. Then, after a slight pause, 13 and 14.

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* Exp. 8/493 denotes that the experiment in question was the eighth of the day and the 493rd since the beginning of the training of this bird. The '20th training day' may be the 20th or the 40th since the beginning of the training; it means that the bird had been actually trained on 20 different single days.

In order to eliminate the possible—though very improbable—influence of smell, the odd grains remaining from the above experiment were now moved to the positions of the even ones. In the next test he actually ate again the odd grains—leaving the grains he had not taken in the previous test. Thus twice the same grains were refused. But this was merely a coincidence. The remaining even grains were again moved to the positions of the odd ones and this time (13/541) he ate them nearly completely (5–13) thus eliminating the possibility of any secondary cue communicated by the sense of smell.

Similar tests were carried out on the following day. In six test experiments (all grains loose, distance 3 cm.) twice he ate the even ones and four times the odd ones with very few mistakes. Now for the first time since the unsuccessful experiment 511, the distances between the grains were again made unequal, but the transition was effected much more carefully than before. The alteration was here only 0·5 cm. (compared with 1·5 cm. in Exp. 511) and the position was as follows: the distance between grains 1–2, 2–3, 12–13 and 13–14 was the original 3 cm.; 3–4, 4–5, 5–6, 9–10, 10–11 and 11–12 was 2·5 cm. and 6–7, 7–8 and 8–9 was only 2 cm. The cock reacted to the experiment (11/552) this time with perfect alternation (eating all odd grains and no. 14 as well). A repetition yielded exactly the same result. The next time he ate the even ones plus grain 3, and then, in three successive trials, the odd grains almost correctly.

During the following days cock 4 did not work well. It was not because of the absence of hunger for he ate well in cage 1 but refused to do it in the run. The bird had a complete rest of 10 days. Then (27th training day, Exp. 1/616) I started with a test experiment—all fourteen grains loose in the standard distance of 3 cm.—and he ate almost correctly 4, 6, 8, 10, 12, 14, 13. This was very remarkable after a complete pause of 10 days. Unfortunately he again showed no 'interest' in succeeding experiments. Three days later (28th day of training) the first test was made in the 6th experiment of the day (6/624). He started with grain 2 but then ate the odd ones (2, 5, 7, 9, 11, 13, 14). The following tests, however, gave quite erratic results. Five more days of complete rest were therefore interposed and I started again with a test experiment (627) (all grains loose). The result was exactly the same as 9 days before in Exp. 1/616. During the next twenty-eight training experiments he ate correctly all even ones, the odd ones being fixed—in Exps. 5, 6, 10, 13, 16, 18, 21, 22, 24, 28 and 29 of the day. In Exp. 15/641 he started wrongly with 1, 3, 5, but corrected himself for the first time by going back and starting now

2, 4, 6, etc. This behaviour, however, must not be over-estimated, as the fixed grains were again distinctly darker on this day in order to make the first experiment with a 'moving lid' less difficult for the bird. As was to be expected, the presence of this new device (speed 5·25 cm./sec.) surprised the cock so much that he did not eat. He looked at the moving lid instead of at the row of grains. When six grains were visible he started eating the even (loose) ones, and when he had finished the last (third) grain the whole row was already visible and was correctly eaten. But this result was of course not produced under the desired conditions. The speed had to be reduced to 2·15 cm./sec., but then he pecked at every grain as soon as it became visible (Exp. 36/662). The grain, actually motionless, but apparently moving relative to the lid, had the same attractive power for the bird as a really moving grain. The experiments were repeated several times but always with similar results, although tests interposed between them (without lid) proved that the bird was then very well able to peck only the loose grains. Of course it was quite hopeless to try the moving lid over a row of 'all-loose' grains. (For more experiments with the moving lid see hen 1.)

The first test of the following day Exp. 1/667 (all grains loose) was a failure (1, 2, 4, 5, 6, 8, 10, 12, 13, 14). No fresh training was made with fixed odd grains, but the test simply repeated (Exp. 2/668), and this time surprisingly the cock ate the even grains perfectly, though he took grains 13 and 9 on his way back. So it was only 'lack of interest' or 'attention' that caused the failure in the first test. In the following test the distances between the grains were increased to 5 cm., with the result (Exp. 670) that the whole row, except grains 5 and 9, was eaten at once. More surprising still in the next test, when the distance was again reduced to 3 cm., all even grains were eaten in correct alternation and none of the odd ones touched. All grains were, of course, loose.

The last mentioned tests may seem to contradict the results obtained with hen 1. But in fact they do not, and we shall discuss the reason for this apparent discrepancy later (see Exps. 634–36 and 690 of hen 1).

Experiments with hen 2

This specimen was very quiet and from the beginning much tamer than all other birds. Her progress in learning was not very quick, rather it was steady. She, too, trained herself to eat darker grains. Small changes in the apparatus upset her as well as the other birds. The first test (all grains loose) was made in Exp. 15/489 of the 16th training day but there were still many mistakes. Even on the 19th day the first tests (Exps. 13/546–16/549) were not very successful, but Exp. 17/550 was a perfect solution: all the even grains were eaten while no odd grains were touched. All were, of course, loose.

Immediately afterwards—perhaps too early—I made a test with unequal distances between the grains (distances identical with Exp. 511 of cock 4). The result was the same as in the case of the cock (nearly all eaten).

During succeeding days the same phenomenon as in cock 4 was observed, namely, that when the first test was unsuccessful (all grains loose) the alternation nevertheless returned without any training. Even a test with unequal distances of the grains varying from 4 to 2 cm. was successful (Exp. 11/615). The distances were: grains 1–2 and 2–3, 4 cm.; 3–4 and 4–5, 3·5 cm.; 5–6 and 6–7, 3 cm.; 7–8 and 8–9, 2·5 cm.; and from 9 to 14 every grain had a distance of 2 cm. from its neighbour. The hen ate 1, 3, 5, 8, 10, 12, 14 and 13, 9, 6, on her way back—making only one mistake in the alternation. This is remarkable as the pecked distances varied here from 8 cm. (grain 1–3) to 4 cm. (grain 10–12 and 12–14), but the gradual variation of the distances is apparently an indispensable help.

On the 29th training day I started the new task of training the bird to eat two grains and to leave the third one. This differs from the scheme used by previous workers where the birds are reported to have selected every third grain. I assumed it would be easier to train them to eat two grains than to leave them. The new training board had therefore fifteen grains of which nos. 1, 4, 7, 10 and 13 were fixed. The distances were again 3 cm.

The first experiment (Exp. 715) gave a very significant result. The following grains were eaten: 2, 5, 8, 11, 14, (15). To a casual observer this would have appeared a successful training to select every third grain, but actual observation of the pecking bird revealed an entirely different solution. The bird started with grain 2 and went on—according to the previously trained alternation—to grain 4. This, however, was now fixed, so the hen pecked 5 and ate it. The successful pecking resulted again in alternation and the hen now pecked grain 7 which was also fixed. Grain 8 was now pecked and eaten and 10 pecked. Grain 10 was fixed, therefore 11 eaten and 13 pecked unsuccessfully. Then nos. 14 and 15 were eaten—the latter, being the last grain, was irresistible. The following scheme shows the reaction of the bird quite clearly:

Pecked	2	4, 5	7, 8	10, 11	13, 14, 15
Eaten	2	5	8	11	14 (15)

This was not a coincidence. Exactly the same response was observed in the following experiments (716–721) with the only difference that the hen twice started with the (fixed) grain 1 which of course made no difference to the final result. Later she occasionally ate some of the remaining loose grains on her way back. Even 2 days later (30th training day) the first five experiments gave exactly the same result. From the 6th experiment onwards the first progress in the new training scheme was observed but during the whole day (47 experiments) no perfect solution was obtained. On the following day for the first time she pecked the right grains but often not in the right order, e.g. she pecked 3, 2 instead of 2, 3 etc. ('inversion').

Exp. 8/783 of the 31st training day gave the first perfect result: 2, 3–5, 6–8, 9–11, 12–14, 15 (on a training board with fixed grains 1, 4, 7, 10, 13) and many more followed during the same and the following day. But the fact that the hen in these successful trials ate rather slowly and sometimes nearly pecked a wrong grain but resisted at the last moment, made me suspicious. When now in Exp. 21/819 a row of all-loose grains was offered the result was a complete failure. She started 2, 1, 3, 4, but then ate all grains till no. 12—leaving 13–15 as she apparently did not 'feel entitled' to eat more than twelve grains. On the other hand, when the training board was used again immediately afterwards, she did not touch the grains 1, 4, 7, 10, 13 in Exps. 820–822. So it was quite clear that the bird had again trained herself to discriminate between different shades. To start darkening the fixed grains again seemed futile. Therefore I now tried a 'figural' help. The loose grains were placed in a line parallel but 1 cm. left of the fixed ones. It was surprising to see how little this new position helped the bird. Even when the distance of 1 cm. was increased to 3 cm. the results did not greatly improve. Exp. 35 of the day gave a positive result and the distance was again reduced to 1 cm. and after a perfect solution (Exp. 41) the grains were placed in one row. The training was continued for 10 days, but without any progress, i.e. as soon as a board was freshly stocked with barley, the results were negative.

As there was no prospect of any success the scheme was now altered to two fixed and one loose grain in a row of fifteen—the grains 3, 6, 9, 12, 15 being loose. This was the original scheme used by Katz & Révész (1909, p. 106), but here, too, I was not able to observe any progress although the experiments were continued until 26 February 1941 (317 experiments in 11 training days). Often a success seemed to be achieved, but the control tests always gave the same negative result. So the attempt to train this hen to select every third grain from a row of loose grain was as unsuccessful as the training to eat two and to leave the third grain.

One could object that this particular hen was now handicapped in her learning ability after her training had been changed twice, and to investigate the possibility I now repeated some of the simple discrimination experiments described by Katz & Révész (1909). She learned after only twelve trials to discriminate between rice and barley (five loose rice grains between six barley grains, glued on cardboard). Several times she pecked the last glued grain for reasons frequently mentioned before but perfect solutions (all loose grains eaten, none of the fixed ones pecked) were very common. Often three perfect solutions occurred in succession. But as soon as all grains were loose the whole (or nearly the whole) row was eaten at once—thus proving again that these quick 'successes' are nothing but a discrimination between loose and fixed grains. Nine days after the last experiment I wanted to see how much of the old alternation training was kept by the bird. A row of loose grains with the original distance of 3 cm. was offered. The result was: all eaten except grain 5, and in a repetition only the grains 4, 5 and 11 were left. On the same day, three more tests with 'all-loose grains' were again a complete failure, and 4 days later (48th training day) the results were exactly the same.

Another hen (no. 1) was able to alternate after a very long pause (20 days) without fresh training. The reason for the bad results in the case of hen 2 was probably the antagonism between the two different schemes of training, and it appeared therefore advisable to interpose a complete rest for 2–3 months in order to wipe out, if possible, the memory of the previous forms of training, and to see whether there would be a distinct saving of time in the relearning of the first scheme (simple alternation). (Compare the debate about relearning between Yerkes (1934) and Spence (1939).)

When, after a pause of 3 months, the experiments were resumed on 10 June, 1941, with an all-loose row, all were eaten, and the same happened when the tests were repeated twice. On a training board, however, good results were achieved.

Four days later the first experiment with a freshly stocked graining board gave a perfect solution as also did Exp. 18. I now tried a test with 'all-loose grains 3 cm. apart. The result was: 3, 5, 7, 9, 11, 13, 12—showing that the alternation was now almost perfectly mastered on the second day of retraining, after an interruption of more than 5 months. (Three training days later a faultless alternation was observed: Exp. 28/1443.)

It was interesting to see what degree of stability the results of the training had now reached, and the tests were therefore continued on the same day. The hen started with the even ones 2, 4, 6, 8, but now—what had hardly ever happened before—she went back to 7 and ate 7, 10, 12, 14, 13. The next test—all loose again—was similar: 2, 4, 6, 8, 7, 9, 11, 13, 14. One has to realize the position of the grains after 8 and 7 had been eaten: in both cases there was a gap of 12 cm. between the grains 5 and 9—and with grain 9 there started a new regular row (9, 10, 11, 12, 13, 14). In Exp. 21/1374 the bird commenced with the even ones and in Exp. 22 with the odd ones of the new row, which actually was only the second half of the original series. In both experiments the temptation to eat the last grain, as usual, was irresistible. One can say that here, though the alternation of the whole row was incorrect, the two halves taken separately were correctly treated.

Experiments with hen 1

Hen 1 was an energetic and voracious bird, always 'willing to work' during the experiments, but in spite of this fact, her progress was slow compared with cock 4 and hen 2. Even on the 11th training day there was no real success, though the fixed grains were distinctly darker than the loose ones. I therefore gave the fixed grains a very dark grey shade by means of a pencil and now she 'understood'. That is, she associated either the brightness of the grains with edibility, or the darkness with inaccessibility, or both. Only seven experiments were needed before the first perfect selection was achieved, Exp. 10 and later Exps. 19, 21, 34, 36, 37, 39 and 48 of the day gave correct results—all even ones eaten and none of the odd ones touched.

On the 13th training day many experiments gave perfect results, but possibly the training was only to discriminate between different shades. When I now used a freshly stocked training board I expected a failure, but the hen pecked only grain 1 (fixed) and then correctly ate all the even ones (Exp. 20/473). Thus with the help of a striking and unmistakable difference of shade this hen learnt to alternate. After 5 more training days she was able to do it even with a row of all-loose grains.

On the same day tests with unequal distances between the grains were introduced. In contrast to the corresponding experiment with cock 4 (552–4) and hen 2 (589–91), but identical with Exp. 615 of hen 2, the distances varied from 4 to 2 cm. Hen 2 had made one mistake, but hen 1 promptly solved the problem by eating all odd grains in perfect order. It is important to realize the difficulty introduced by the differences in the pecking distances, viz. 8 cm. (grains 1–3) and 4 cm. (grains 9–11, 11–13). At the same time the gradual diminution of these distances provided an indispensable help as before in the case of other birds. The success of the bird is not lessened by the fact that she ate the grains 14, 12, 10 and 8 on her way back (Exp. 21/629). To exclude any doubt the same scheme was repeated in the following tests. In the first one, grain 3 (now of course loose) was missed by the bird and thrown near grain 6. She therefore ate: 1, 3 (3 → 6), 6, 8, 10, 12, 14, 13, 11, 9. The result of the second test was again perfect (all odd ones and 14, 12, 10, 8, 6 afterwards). When now the normal distance of 3 cm. was doubled by offering a row of loose grains with a distance of 6 cm. the hen started with the grains 2, 4 and then ate all the remaining ones.

On the 19th training day I used a board with distances of 4·5 cm. between two neighbouring grains (distance between fixed grains = 9 cm.). Hen 1 solved the problem perfectly (Exps. 634 and 635). In the next test all fourteen grains were loose—the distance still 4·5 cm.—and again the hen correctly ate all odd ones in the right order. This good result encouraged me to try a 5 cm. spacing, and in order to avoid too long a row unequal distances were now introduced varying from 5 to 2·5 cm. (Exp. 637: 1–2, 2–3 and 3–4 = 5 cm., 4–5 = 4·5 cm., 5–6 and 6–7 = 4 cm., 7–8 = 3·5 cm., 8–9 and 9–10 = 3 cm., 10-11, 11-12 and 12-13 = 2·5 cm., 13-14 = 3·5 cm.). Although the pecked distances varied from 10 cm. (1-3) to 5 cm. (11-13), the hen ate all odd ones in perfect order (and 14, 12, 10 on her way back). In a repeat test (638) the result was even better: 1, 3, 5, 7, 9, 11, 13, 14.

These two experiments seem to contradict the result of Exp. 670 of cock 4, who had failed in a similar trial. One could object that hen 1 had the benefit of fixed odd grains, but this applies only to Exp. 634/635, while in the case of the following three decisive tests of hen 1, all grains were loose. In addition, one could say that the distance in the case of cock 4 was 5 cm., while here it was only 4·5 cm.—but in Exp. 637 the first four grains were 5 cm. apart. The decisive fact is, in my opinion, that in the case of hen 1 the distance of 4·5 cm. was offered in the first experiment of the day, while cock 4 in the corresponding test had eaten immediately before, three times in succession, rows with 3 cm. distance between grains. In order to solve this problem, 10 days later these experiments were repeated with hen 1 (28 January 1941, Exps. 686-690). This day's tests started with four perfect results. (Even ones eaten in Exps. 1, 2 and 4; odd ones in Exp. 3.) When the distances in Exp. 5/690 were suddenly extended from 3 to 5 cm., the hen ate the whole row of odd and even ones, one grain after another, and thus reacted in exactly the same way as cock 4 in his Exp. 670. Even more significant perhaps is the fact that the reduction of the distance back to 3 cm. resulted in a perfect alternation.

Thus the observation, made repeatedly, that a sudden alteration of more than 50% of the distances inhibits the alternation was again confirmed.

We have now to look back to Exps. 637-8. Here the distance varied from 5 to 2·5 cm., but perfect alternation was achieved in both cases. It remained to be seen how the bird would behave when the order was reversed. In the following test (639-40) the distances were therefore increased (apart from the first one) (3·5, 2·5, 2·5, 2·5, 3, 3, 3·5, 4, 4, 4·5, 5, 5, 5 cm.). During the first test (all grains loose of course) the hen chose 1, 3, 6, 8, 10, 12, 14, 13, 11, 9 with the pecked distances 6, 8, 6·5, 8, 9·5, 10 cm., but in the second she 'readjusted' herself and performed a faultless alternation of the odd grains (pecked distances now 6, 5, 6, 7·5, 8·5, 10 cm.).

I now wished to discover whether this hen would, after 630 experiments, be able to adjust her behaviour on a training board, where, in contrast to the usual arrangement, the even grains were fixed and the odd ones loose. I used an ordinary freshly stocked training board on which the first loose grain was laid in front of the first fixed grain instead of between the first and second fixed grain. There were two possibilities—she would either start with the even ones (now fixed) and would have to abandon them and start afresh (see cock 4, Exp. 641), or she would start with the odd ones (now loose), but in this case she would possibly find trouble in the case of the last 'irresistible' grain 14—now fixed. This actually happened and the hen ate (or pecked respectively) very quickly 1, 3, 7, 9, 11, 13, 14, 14, 14. It is not clear why grain 5 was left, but the explanation of the quite unusual fact that grain 14 was pecked three times is that this grain had always been loose in previous training experiments *and* all-loose tests. It will not be misunderstood (see introduction) when I say that the hen 'felt entitled' to have the last grain and was consequently very persistent in her effort and afterwards 'disappointed' and puzzled. Marold (1939, p. 203) had a similar experience in the case of 'premature frightening' of his budgerigars. These birds had learned to eat six grains from a heap of 10-15, and were used to being chased away when they attempted to eat a seventh grain. When Marold chased the bird after it had obtained only four grains it was extremely surprised and did not want to leave the place—it 'stood its ground', as it were.

On the 20th training day experiments with a 'moving lid' were started. After seven training experiments (correct results in Exps. 3 and 7) the lid was used in Exp. 8/653. The first four grains (two fixed, two loose) were uncovered, and when the hen entered the run the movement of the lid (speed 1 cm./sec.) begun. She pecked and ate only the visible loose grains and went back to cage 1, as the movement of the lid was too slow. When the speed was increased to 2·15 cm./sec. the hen behaved in the way described in the case of cock 4. She pecked every grain—fixed and loose ones—as soon as they became visible. In later experiments she often pecked only four to eight times and then waited until the whole row was uncovered and visible. She then selected the loose grains which were purposely made somewhat darker and when, without pecking, she had followed the moving lid until the end of the row, she sometimes ate the loose grains backwards: 14, 12, 10, etc. The experiments were repeated for several days with similar results. Thus it was again impossible to find out whether any alternation was possible without the help of the visible 'pattern' of the row.

During the following days the hen developed more and more the habit of eating in 'both directions', which means that after a successful alternation of the odd or even grains she ate the remaining grains on her way back. This made it sometimes difficult or even impossible to judge the test properly, especially in experiments with rows of unequally spaced grains. It was quite easy to prevent the hen by covering the row with a piece of cardboard as soon as she had finished eating in one direction. The cardboard, just long enough to cover the row, was put alongside the row before the test started, and at the right moment, by means of a wooden handle outside the run, it was pushed over the remaining grains.

Further experiments were again devoted to the problem of 'readjustment' to suddenly altered conditions. On the 30th training day, after perfect alternation of all-loose rows with a distance of 3 cm. (pecking distance 6 cm.), the distance was reduced to 2 cm. The first result was now 2, 5, 9, 11, 13, (14) with pecking distances of 6, 8, 4, 4 cm., but in the following tests the bird adjusted herself almost perfectly to the reduced distance of 2 cm. by eating (2), 3, 5, 7, 9, 11, 13 in Exps. 33/814 and 34/815. Then on reversion to the old distance of 3 cm. the alternation was upset, but in the following test, 36/817 (again 3 cm. distance), a secondary spontaneous readjustment effected a faultless selection of all odd grains.

Similar observations were made on offering the grains for the first time not in a straight line, but in the form of a sine curve (39 cm. long) of total amplitude 2 cm. and wave length 24 cm.

The experiments started on the 32nd training day with normal test rows (straight line, 3 cm. distance) which gave nearly perfect results. The first curved line (Exp. 5/871) gave the expected result (eaten 1, 3—then all grains from 4 to 14), although the alteration of the distances, compared with the previously eaten straight line, was negligible—only about 3·1 cm. compared with 3·0 cm.—which was probably not even perceived. Two tests with straight lines gave (Exp. 6) a nearly and Exp. 7/873 a perfect selection of all odd grains. The succeeding tests with rows in a sine curve showed a slight improvement, and the last two experiments of the day (21–22) showed again a nearly perfect alternation (1, 2, 3, 5, 7, 9, 11, 13, 14—leaving 4, 6, 8, 10, 12). 'Spontaneous adjustment' to a rather different condition was therefore demonstrated.

On the 33rd training day the effect of 'shifting' of the row was again examined. At first the row (of course all grains loose) was moved on the teak board 21 cm. to the left, and the hen kept the alternation. Even at 50 cm. a row was eaten in nearly perfect alternation: 2, 1, 3, 5, 7, 9, 11, 13, 14. In the next test the rows of grains were placed not on the teak board, but on the concrete of the table—in a line parallel and only 12 cm. away from the usual position—but now the hen ate nearly all without hesitation. A repetition gave the same result. When now in the next test (14/909) the row was on the teak board again the alternation returned at once (1, 3, 5, 7, 9, 11, 12, 13, 14), at least for the first six pecking reactions.

On the 34th training day 'memory tests' were carried out. The hen had not been used for a full week. The first test of the day (1/918) with an all-loose row with normal distances (3 cm.), gave a satisfactory, though not perfect, result: 1, 4, 6, 8, 10, 12, 13, and then the hen was stopped to prevent her from eating backwards. The next four tests, however, gave completely negative results.

Twelve days later the results were again completely negative. But there was still the possibility that some of the training was left. So 3 days later I started again with an all-loose test and the hen performed a perfect alternation (all odd ones eaten, after grain 13 stopped, Exp. 1/938). Repetitions of this test on the same day were less successful. Test 938, however, is remarkable, as such a perfect result had not been observed for more than a month—especially as there had been in the meantime no real training—and no training at all since the negative results of the 34th and 35th training day. These last observations show how fallacious it would be to conclude from a few adverse results that the bird is not able to solve the task. A perfect alternating selection is most unlikely to be achieved by pure chance, but if the hen is 'willing' to do it, she can master this problem even after several weeks without any new training.

It is unnecessary to report in detail the experiments with the other birds, as they yielded no different results. Hen 3, as already stated, was used for checking the results of previous papers, and so was cock 5. The latter learnt very quickly—after only twenty trials at the second day of training—to select without mistake every second grain of rice from a light board (weight about 360 g.). This was used as he several times pecked loose the grains glued to cardboard—a difficulty of which W. Köhler (1915) had previously noted. Exps. 22, 25, 26, 30–33, 39, 42–43 were again all faultless; but when in Exps. 45/71 all grains were loose, the whole row, as I expected, was eaten. The same bird was used for the problem of selecting every third grain. He trained himself, as hen 2 had done before, to secondary cues. When these were cut out, he failed.

Cock 10 showed no difference from other birds in his progress of learning.

Jungle fowl were extremely shy and suspicious. They had to be trapped each time in a shifting cage for the experiments, and it took several days before they dared to eat in the run. They never ate the whole row at a time, but after eating three to five grains looked round as if to see if danger were imminent. The cock (6) learned more quickly than the domesticated birds to distinguish loose and fixed grains by shade, and then made very few mistakes. A board freshly stocked with grain, however, gave a completely negative result (23/43). It is, of course, quite impossible to draw any conclusions about his learning ability from this fact, as it was much too early to expect more. No essential differences in learning from domestic fowl were observed.

DISCUSSION

The experiments described in this paper show that fowls are able to perform an alternation, i.e. to select every second of a number of equidistant loose grains arranged in a straight or curved line. In addition, the distance between the grains can be varied to a certain extent. The learning of the alternation, however, is effected in a different way than was previously assumed. Earlier papers, dealing with the same problem, maintained that birds were able to alternate after only 15–30 training experiments, and even report the ability to alternate in an irregular way, e.g. to select grains 2, 8, 4, 10, 6 instead of 2, 4, 6, 8, 10. This would be convincing if these experiments were supported by control tests in which all grains were loose, but there are no reports on such tests in these papers. This is all the more surprising as their authors have stressed the fact 'that apart from the glueing method control tests are necessary in which all grains should be loose, and only if the experiments are successful under these conditions can one guarantee the results' (Révész, 1922, p. 471). We are therefore bound to assume that these birds learned merely to discriminate between loose and fixed grains. Consequently, the conclusions drawn by Révész (1922, p. 470), especially his assumption that the distances between the grains were irrelevant for the ability to select every second grain, are without foundation. Nevertheless, the results of Révész's experiments have been reported as established facts in many papers dealing with similar problems, though other authors have already severely criticized his technique (Bingham, 1922; Warden & Baar, 1929).

In contrast to previous workers I found that 500–600 training experiments were necessary for the birds to learn a true alternation in rows in which all grains were loose. The real difficulty was to make the birds 'understand' what was actually required from them, and in order to achieve this the temporary use of crude secondary cues was often indispensable. This detour training has also been found necessary in experiments dealing with similar problems, e.g. the 'figural help' given to birds in experiments by Arndt (1939) and O. Koehler and his collaborators (1935–6). In my experiments differences in shade proved to be more effective than figural help.

Many readers will be surprised to realize that the presence of easily recognizable secondary cues in training experiments never yielded 100% correct solutions for any length of time. Apart from lack of attention, there is certainly often a lack of 'interest' in the correct performance of alternation. The discrimination of loose and fixed grains was no doubt often very easy for the bird, but in spite of this fact it did not act accordingly as it was of so little importance for it. Neither was there any punishment in the case of a negative choice—except that a maximum of seven grains were pecked in vain—nor any reward in the case of a correct choice—except that a maximum of seven grains were available. This would not particularly influence the bird, however, as it was allowed to eat four to six times this amount between every two experiments. In addition, the bird had to be kept between two physiological extremes: when it was very hungry it was clearly inclined to peck at every grain lest it should miss one, and when it was not hungry it would not always trouble to find the few loose grains. From this point of view it is certainly surprising that the birds were able to learn the somewhat unnatural task of performing an alternation.

This is all the more clear if we look at the results of the very few corresponding investigations carried out with other animals. Though neither the alternation in a temporal maze nor in the 'multiple-choice' apparatus can be strictly compared with the experiments described in this paper, it is significant that, in contrast to many successful experiments in the spatial maze, the alternation tests in the temporal maze (which alone represent a true alternation) gave very poor results (Hunter, 1928; Gellerman, 1931; Karn, 1938). The alternation in 'multiple-choice' experiments was, apart from the hitherto unsolved middle-box problem,* the most difficult task for all the animals investigated (Coburn & Yerkes, 1915; Yerkes & Coburn, 1915; Yerkes, 1916, 1934, etc.). It has been achieved only by two pigs, one of two monkeys and one of four chimpanzees.

The training of the fowls here described was actually not to alternate, but simply to eat the grains in the position of the even numbers of a series, as the odd ones—with the few exceptions detailed above—were always fixed during the training. Thus it is remarkable that the birds learnt to alternate independently of whether they started with the first or second grain in the row. Consequently, in successful tests with all-loose rows the birds ate all odd or all even grains, and there was no proof of a predilection for either of them, even if they started afresh in the middle of an interrupted row where a gap produced, as it were, a new row of grains (e.g. Exp. 1374/76 of hen 2).

From a theoretical point of view it is necessary to realize that these experiments differ entirely in method from those in which a simple discrimination between two 'stimuli' is achieved by the animal. In the latter case it is of the utmost importance to avoid helping the animal by means of minute movements given unwittingly by the experimenter. This danger proved non-existent, as the pecking reactions took place so quickly that it was often difficult for the observer to follow the movements of the birds, and it was obviously quite impossible to give any help in selecting the right grains. This was checked by observers who had never seen the birds before and did not know what was expected from them—but the results remained unchanged.

One difficulty of experimental procedure should be mentioned. While in simple discrimination tests the results are either positive or negative, it is obvious that here a great number of intermediate solutions are possible. Only such tests could be considered as entirely successful in which the birds had eaten all the loose grains and had pecked none of the fixed grains during training experiments, or had performed a true alternation in a crucial test in which all fourteen grains were loose. This shows the difficulty in judging the results, and the even greater difficulty in estimating the progress of learning. Using arbitrarily a criterion of five or ten perfect tests in succession would be a great mistake and might lead one to underestimate the possible achievements of the birds.

Another difficulty for the experimenter was to find how soon and how often tests with all-loose rows could be offered between the training experiments with partly fixed grains. It is of course quite impossible to work according to a fixed plan—in fact nothing could be more detrimental to the successful progress of the experiments. It is often a matter of intuition to decide whether on a certain day the birds will 'stand' some more tests without endangering the training. In the beginning of the experiments I was frequently over-cautious in thinking it necessary to return to the training as soon as some tests with all-loose grains gave unsatisfactory or irregular results. Later I realized that in such cases the training is not destroyed, but merely 'suspended' by the bird, and very often the alternation returned without any fresh training. Gradually I learned which conditions were likely to induce the bird to abandon the training habit. For example, doubling all the (equal) distances between the grains (all loose of course) had in every case the effect of making the bird abandon the alternation at once. This, however, could be prevented by gradually introducing the extension of the distances.

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* For the difficulties and possible errors in the interpretation of apparent solutions of this problem see Honigmann (1942).

The use of rows with unequally spaced grains showed that sudden alterations suspended the alternation, while extensions or reductions of the distances, gradually introduced in one and the same row, made no difference. The fact that the pecking distance in one and the same row could be doubled in this way seems to me very remarkable. If a bird is able to perform a perfect alternation in a row of loose grains, the correct pecking distances of which vary from 8 to 4 cm., it shows without any doubt that the pecking distance does not play any decisive role. Of course this differs from Révész's (1922, p. 470) untenable claim that the distances between the grains in the row have no influence whatever on the 'quality' of the alternation.

In the case of an alteration in the usual position of the row of grains the results were as follows: a shifting of the whole row by 30 cm. was the maximum, though often smaller distances adversely affected the results of the tests. Again, when introduced gradually, much larger distances did not upset the alternation.

The original aim to eliminate any 'pattern' effect by means of a moving lid which made only parts of the row of grains visible proved impracticable as the birds were unable to resist the 'temptation' of a moving grain. Actually the grains were stationary, but their increasing distance from the moving lid was sufficient to produce the effect of a real movement. Benner (1938) had a similar experience during experiments with fowls on the discrimination between real grains, and grains projected by means of an epidiascope on a frosted glass pane from below. The birds learned to recognize the difference in a short time, and after 10 days did not look at the projected grains at all. But when Benner now moved the real grains so that the images on the frosted pane moved as well, the birds pecked them again. Possibly a method employing a punishing device in the case of a negative choice would be necessary in this type of experiment, but the disadvantages of such a method are obvious.

The same facts which made it impossible to exclude the training to a certain pattern prevented me from performing any decisive tests on the effectiveness of rhythm in these experiments. That a temporal rhythm alone is not decisive is proved by several observations—perhaps in the most convincing way by the tests of hen 2 mentioned above (p. 147). On the other hand, it is obvious that the temporal sequence of the pecking movements and especially its uniform speed has a certain influence on the correct alternation, as an interruption often, though not always, produced a transition from the even to the odd grains, or vice versa, and caused other mistakes. The observations of hen 2 during the fresh training to leave every third grain after she had learned alternation were highly illuminating. They cast an interesting sidelight on the debate between Bierens de Haan (1935) and Koehler & Wachholtz (1936) over the question whether a bird is capable of performing a 'swallowing rhythm' instead of a 'pecking rhythm'. An alternation can be effected by two quite different training actions: either to eat, or to leave every second grain. It does not matter that the results of both are identical in the case of a simple alternation. For this bird the eating of every second grain was decisive, as hen 2 made use of the alternation only after a successful pecking, and thus the unexpected effect—eating grains 2, 5, 8, 11, 14—resulted.

SUMMARY

1. By glueing the odd numbers of a row of grains to heavy teak boards fowls were trained to perform a true alternation, i.e. to peck every second grain from a standard row of which all fourteen grains were loose. This was achieved after 500–600 training experiments in 17–19 training days. No punishment was used in this method.

2. Claims by other authors that a training to a true alternation can be achieved in a much shorter time could not be confirmed. As such results are not supported by control tests in which all grains were loose, it is probable that in these cases the birds have learnt only to discriminate between fixed and loose grains. This also applies to the reported training to select every third one from a series of grains.

3. Although the actual training was effected by preventing the birds from eating the odd grains of the row, the result was nevertheless independent of whether the birds started with the first or the second grain. Rows of odd grains and rows of even grains were eaten without any preference.

4. A sudden increase of the distances between the single grains induced the birds in all cases to abandon the alternation, but it returned without any fresh training when the usual distances were used again. A gradual extension or reduction of the distances, however, did not influence the alternation.

5. A similar result was obtained when the distances between the grains in one and the same row were unequal. Here, too, the alternation was kept when the distances increased or decreased gradually. In this case the correct pecking distances could have the ratio 1 : 2 at both ends of the same row thus proving again a high degree of independence of the absolute pecking distance.

6. In most cases a modification of the experimental conditions (e.g. a different colour of the ground surface, or a curved line of grains instead of a straight line) gave negative results, but a 'spontaneous readjustment' resulting in alternation took place frequently without any fresh training.

7. The intended use of a moving lid covering parts of the row of grains in order to avoid a training to a figural pattern or to a temporal rhythm proved impracticable as in this case the birds did not resist pecking at every single grain as soon as it became visible. Apparently the moving of the lid relative to the actually motionless grains had the same effect as really moving objects which have proved to be highly attractive to many birds.

8. The pecking rhythm had no decisive influence on the alternation, but any change of the uniform speed of the pecking movements definitely facilitated mistakes.

9. After the successful training to alternation, boards with one fixed and two loose grains (1, 4, 7, 10, 13 fixed) were used for the unsuccessful training to leave every third grain untouched. The result was that the loose grains 3, 6, 9, 12 were not pecked (see p. 147). This proved that only the successful pecking acts were 'counted' by the bird.

10. Relearning of alternation after an interruption of more than 5 months resulted in a considerable saving of time: instead of 19 training days (550 experiments) alternation was almost perfectly mastered again by one bird at the second training day (29 experiments) and completely at the fifth training day (99 experiments).

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* The original paper by Ohtsuka was not available. Its contents are quoted from a review in *Psychological Abstracts*.

SECOND REPORT ON A TEST OF McDOUGALL'S LAMARCKIAN EXPERIMENT ON THE TRAINING OF RATS

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The late Prof. McDougall's four 'Reports on a Lamarckian Experiment' are likely to become a classic of experimental biology, as being the most sustained attempt up to the present to demonstrate the reality of Lamarckian inheritance in a particular instance. Although, as will appear from this account of our own experiment, we do not believe that it will take its place in the history of biology as a successful attempt, we take this opportunity of acknowledging our appreciation of his nearly twenty years' devotion to this very laborious and exacting experiment.

Our first report (1935) on a test of McDougall's experiment dealt with the first five generations; we now offer a second report, covering twenty generations. Since the publication of our first report, McDougall published his fourth report, and Crew has published the full account of his own similar experiment, now brought to an end, of which only a preliminary report was available in 1935.

The nature of the experiment is now so well known that it is unnecessary to redescribe it in detail. Briefly, the apparatus consists of a tank full of water, divided into three parallel passages by two partitions which stop short of the far end of the tank. At that end, therefore, the passages communicate. From the near end of each of the side passages a wire ramp leads to a platform above the water level. The rat is placed in the water at the near end of the central passage. In its search for a way to escape from the water it swims down the central passage, turns right or left into one of the side passages, and swims back along this to the ramp at the end of it and climbs out. Behind a sheet of ground glass at the back of each ramp is a low-power electric lamp, which shines through the ramp down the whole length of the passage, illuminating also its communication with the central passage, so that the rat at its starting point in the central passage can see which of the two side passages is illuminated. The rat is given six trials a day (except for the first 5 days of training, when, unlike McDougall, we give only four trials) with the left and right passages illuminated alternately. The ramps are connected with an alternating current in such a way that the current is thrown into whichever ramp is illuminated, but not into the other. The rat can therefore escape from the tank by either gangway, but if it chooses the illuminated ('bright') one it does so at the expense of an electric shock. The rat has to learn always to escape by the dim gangway, irrespective of whether this is on the right or left. Facility in learning the task is measured by the number of errors, that is to say, number of escapes* by the bright gangway, made by the rat before it learns to avoid this and always chooses the dim one. The number of errors made is therefore the same as the number of shocks received. A rat is held to have learnt the task as soon as it has made twelve consecutive correct runs. Further details of the apparatus and training procedure are set out in our first report, and have not been changed since. The various factors which influence the rate of learning are also discussed in that report, especially those which appear to be responsible for the great variation in the number of errors made by individual rats even of the same litter.

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* Strictly speaking, attempted escapes. Occasionally a rat makes contact with the electrified ramp, but instead of climbing it, turns back on receiving the shock, swims to the other exit and escapes by it. This very rarely happens except during the first 4 or 5 days of training. This is recorded as an error.

The first report gave the results of two independent lines of rats; since then we have continued with only one of these lines, line A of the first report. All the rats of this line are descended from a single pair of albino rats of Wistar origin. The first generation obtained from this pair (which was not trained) was divided into two groups, one of which (five rats) was trained and became the ancestors of the trained line; the other group (four rats) was not trained and became the ancestors of the control line. The two lines have been bred parallel with each other, and under the same conditions. In each generation, the required number of rats in the trained line are trained, and mated as parents of the next generation. In the control line some of the litters produced are not trained, but are kept as parents of the next generation. Other litters of this line are trained, to provide controls to the same generation of the trained line. These trained controls are, of course, not used for breeding. In this way each generation of the trained line is tested against an approximately equal number of trained controls, differing from the trained line only in the fact that their ancestors were not trained.

Our system of mating ensures that the number of errors made by any rat in the trained line does not influence its chance of becoming a parent. As described in our first report, the few rats which have not learnt after 52 days of training (by which time they are 80 days old) are given 'special training'. These slow learners are invariably rats which, early in training, have formed the habit of using exclusively either the right- or left-hand exit passage, whether this is illuminated or not. Consequently they receive a shock on every alternate run. As the rats are given four trials a day for the first 5 days, and six a day thereafter, by the end of 52 days they have had 302 trials, and have received approximately 151 shocks. 'Special training' consists in forcing them for a few times to use the unfamiliar exit. After this they invariably learn to use the dim passage, either at once or after a very few days. Thus even the slowest learners have completed their task before any rat is mated. In fact, the reason for giving 'special training' to these rats is to ensure that slowness in learning shall not result in later age of mating (for they cannot be mated before they have learnt) and therefore in diminishing their chance of becoming parents of the next generation. The number of rats which received this 'special training' can be seen from Table 2, where they are shown by the letter S.

The rats are paired without any reference to their training scores; in fact, the pairs are formed by drawing lots, brother-sister matings being, however, avoided in both lines, except in a few cases where the total number of rats available was small. Not all the rats mated become parents of the next generation, for many of the matings prove infertile, and others only produce litters after the number of young required for the next generation has been obtained. It is our usual practice to reduce large litters to six; the number of litters required to produce a generation of fifty is about ten. Although our system of mating does not discriminate between rats in regard to their training scores, it is always possible that by chance the rats which have become parents may not be a representative sample of the total population. This can only be ascertained by comparing the training scores of those rats which became parents, with those of the total population. The figures in heavy type in Table 2 are the rats which became the parents of the next generation. It will be seen that there is a total of 193 parents (trained line), and that these form a representative sample of the 702 rats in that line. The median score of these 702 rats is 24, and the percentage of rats learning with fewer than 10 or more than 100 errors is 15·2 and 6·7 respectively. The corresponding figures for the group of 193 parents are 24, 12·4 and 6·2. Thus there has been no accidental selection of rats with high or low scores.

RESULTS OF THE EXPERIMENT IN RELATION TO LAMARCKIAN INHERITANCE

McDougall measured the performance of each generation of rats by the mean number of errors made by that generation, and by the scores of the best and worst rats. Crew used the same measures, with the addition of the median.

Table 1. *Distribution of number of errors made (shocks received) by individual rats in the twenty generations; i.e. in the trained line 107 rats made 0–9 errors, etc.*

No. of errors	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89
Trained line	107	158	170	105	55	22	11	17	6
Control line	99	133	163	107	53	16	18	6	6
Totals	206	291	333	212	108	38	29	23	12

No. of errors	90–99	100–109	110–119	120–129	130–139	140–149	150+	Total no. of rats
Trained line	4	4	0	2	2	1	38	702
Control line	7	4	4	3	4	3	44	670
Totals	11	8	4	5	6	4	82	1372

The arithmetic mean is an unsatisfactory measure of the central tendency of a group in which the distribution is as unsymmetrical as the distribution of number of errors made by the rats in this experiment (Table 1). Indeed, the use of this measure becomes impossible in our case, for the distribution includes a final class (the rats which, having failed to learn after 302 trials, received 'special training') in which the number of errors which the members of that class would have made, if training had continued in the same way, is unknown. For this reason we have used three measures for comparison: the median (Tables 2, 3), together with the percentage of rats learning with fewer than ten shocks, and the percentage requiring more than a hundred shocks before learning (Table 3). The data for calculating any other measure of the collective performance of each generation or group are available in Table 2, which gives the individual score of every rat.

In order to obtain larger groups, Table 3 has been constructed, in which the generations have been grouped in fours. As, however, no rats were trained in the first generation of the control line, the first group contains only generations 2, 3 and 4 of this line.

An examination of the medians in Table 2 discloses no evidence of a progressive reduction in the number of errors made by the trained line as compared with the control

Table 2. *Showing the number of errors made (shocks received) by each rat, and the median number of errors in each generation. In the trained line, the rats which became the parents of the next generation are in heavy type. The total number of rats in the trained line is* 702, *in the control line* 670

Generation		No. of rats	Median	No. of errors made by each rat
1	T.	5	58	**32, 50, 58,** 70, S
	C.	—	—	—
2	T.	15	40	**21, 24, 28,** 28, **34, 38, 40, 40,** 42, 46, 54, 71, **88,** S, S
	C.	7	36	24, 30, 35, 36, 36, 37, 56
3	T.	25	41	11, **14,** 20, 25, 26, 30, 32, 32, 35, 36, 38, 38, **41,** 42, 42, 51, **54,** 54, 54, **58, 69, 98,** 121, S, S
	C.	24	46	17, 20, 22, 27, 28, 29, 32, 33, 34, 39, 42, 45, 47, 53, 65, 67, S, S, S, S, S, S, S, S
4	T.	19	31	**18,** 18, **19, 20,** 23, 24, 24, 25, 27, **31, 36,** 51, 62, 65, **81, 123, 136,** S, S
	C.	23	34	12, 15, 18, 18, 21, 27, 28, 29, 29, 30, 31, 34, 36, 42, 45, 56, 72, 78, 80, 94, 134, 143, S
5	T.	22	44·5	2, 21, **24,** 25, 27, 31, 31, 34, 35, 39, **44, 45,** 48, 60, 60, 67, **79, 87,** 99, 142, **S, S**
	C.	20	36·5	17, 23, 28, 29, 29, 32, 32, 35, 35, 35, 38, 40, 43, 45, 48, 49, 50, 52, 122, S
6	T.	22	32·5	18, **19,** 20, 20, **24, 26,** 29, 29, 29, **30,** 32, 33, **37, 41, 43,** 43, 45, 56, 70, S, S, S
	C.	22	24	3, 5, 8, 9, 13, 13, 17, 19, 20, 22, 23, 25, 27, 27, 28, 30, 31, 33, 47, 66, 97, S
7	T.	31	29	**0,** 3, 16, 16, **18,** 18, 19, 19, 19, 23, **24, 26, 27,** 28, 28, 29, 29, **32, 38,** 39, 41, 41, 42, 44, 56, **84, 94,** 105, **S, S, S**
	C.	33	30	8, 9, 11, 15, 16, 21, 21, 21, 24, 24, 25, 27, 28, 28, 29, 30, 30, 31, 31, 31, 32, 35, 40, 44, 50, 63, 63, 65, 90, 94, 100, 102, S
8	T.	41	34	1, 6, 8, 10, 17, 18, 19, 19, 20, 22, 24, 26, 26, 30, 30, 30, 31, 33, 33, 34, 34, **35,** 38, 41, 42, 42, 47, 51, 55, 64, 74, 75, 76, 78, 81, **S, S, S, S, S, S**
	C.	16	24	3, 4, 6, 13, 17, 18, 21, 24, 24, 27, 28, 31, 32, 38, 46, S
9	T.	12	14	**1, 2, 2,** 5, 6, 12, **16,** 20, 21, 25, 28, **65**
	C.	47	28	4, 12, 14, 15, 17, 18, 19, 20, 21, 21, 22, 22, 23, 23, 23, 24, 25, 25, 25, 26, 26, 27, 27, 28, 28, 29, 31, 31, 31, 34, 36, 36, 39, 40, 40, 41, 42, 44, 47, 59, 60, 65, 69, 76, 124, S, S
10	T.	13	19	2, **16, 18, 18, 19, 19,** 19, **21, 26, 27, 37, 44,** 131
	C.	37	43	12, 14, 15, 17, 19, 20, 21, 22, 25, 25, 27, 28, 32, 33, 34, 36, 41, 42, 43, 44, 47, 47, 47, 49, 50, 50, 59, 61, 67, 68, 85, 89, 113, 138, S, S, S
11	T.	44	28	5, 8, 8, **10, 12,** 15, 16, 16, 16, 17, 20, 20, 21, 22, 22, **24,** 24, 24, 25, 25, 25, **28, 28,** 28, 28, **29,** 29, **30,** 30, 30, **33, 34,** 34, 36, **37, 38,** 38, 38, 41, **42,** 45, 46, 53, 68
	C.	60	25·5	5, 6, 6, 6, 8, 10, 10, 11, 13, 16, 16, 18, 18, 18, 18, 19, 19, 20, 20, 20, 21, 22, 22, 23, 23, 23, 23, 23, 25, 25, 26, 26, 27, 27, 27, 27, 27, 29, 29, 31, 32, 32, 33, 33, 34, 37 38, 40, 40, 41, 44, 44, 47, 59, 59, 72, 73, S, S, S
12	T.	36	32·5	3, 14, **20,** 20, 20, 21, 21, 23, 24, 24, 26, **28,** 28, 29, 30, 31, 32, 32, 33, 35, **39,** 42, **43,** 43, 45, 57, 60, 70, 72, 75, **76, 77,** 108, S, S, S
	C.	33	22	3, 3, 3, 4, 5, 6, 6, 7, 13, 13, 13, 17, 19, 20, 21, 21, 22, 22, 24, 25, 25, 26, 27, 27, 28, 35, 35, 36, 43, 63, S, S, S
13	T.	38	24·5	2, 3, 4, 5, 8, **9, 10,** 10, 10, 10, **12, 14, 17,** 17, 18, **19, 19,** 19, 24, 25, 27, 27, **28,** 30, 31, 33, 35, **37,** 39, **42,** 43, **79,** S, S, S, S, S, S
	C.	11	31	10, 10, 24, 26, 30, 31, 36, 38, 40, 141, S
14	T.	50	9·5	1, 1, 2, 2, 2, 2, **3,** 3, 3, **4, 4, 4,** 4, **5,** 5, **6, 6,** 6, **7, 7,** 7, **8, 8,** 8, 9, 10, 11, 12, 13, 13, 13, 14, 14, 15, 16, 20, 22, 22, **23,** 25, 25, **26,** 26, 26, 30, 38, 39, 46, 47, 47
	C.	37	19	3, 3, 4, 5, 5, 5, 5, 6, 6, 7, 7, 9, 11, 13, 14, 15, 15, 18, 19, 20, 21, 22, 25, 25, 26, 26, 29, 30, 31, 32, 32, 33, 34, 34, 38, 41, 47
15	T.	49	17	**1,** 3, 4, 5, **6,** 7, **8, 8,** 8, **8,** 10, 10, 11, 11, **12,** 12, 12, **14, 15,** 15, 15, **16,** 16, 17, 17, 17, 18, 18, 20, 20, 20, **21, 24,** 24, 24, 28, 28, 28, 28, **29, 30,** 31, 32, 37, **44,** 49, **62,** 79, S
	C.	50	26·5	4, 5, 5, 6, 6, 7, 8, 9, 11, 11, 13, 13, 13, 16, 16, 17, 18, 19, 21, 22, 23, 25, 25, 25, 26, 27, 28, 29, 29, 30, 30, 30, 32, 32, 32, 36, 37, 37, 37, 38, 39, 41, 44, 46, 49, 54, 65, 78, 87, S
16	T.	50	19	3, 3, **4,** 4, **5,** 5, **6,** 9, 10, 11, **12, 12, 13, 13,** 13, 13, 13, **14,** 14, **15, 17, 17,** 17, **18, 19,** 19, 19, 19, 19, 19, **21, 22, 22,** 22, 22, 23, 24, 25, 27, 27, 31, 32, **36, 36,** 39, **41,** 44, 70, S, S
	C.	50	17	2, 2, 2, 4, 5, 5, 6, 6, 6, 7, 7, 9, 9, 9, 9, 10, 10, 10, 12, 12, 13, 13, 14, 15, 17, 17, 17, 18, 18, 19, 21, 21, 23, 25, 25, 26, 26, 26, 28, 30, 30, 33, 34, 38, 61, 67, 95, 106, 131
17	T.	80	19	0, 0, 1, 1, 2, 2, 2, 2, 3, 3, 4, 4, 4, 5, 6, 7, 7, 7, 8, 9, **10,** 10, 10, **11,** 11, 11, 12, **13, 14,** 14, **15,** 15, 16, 16, 16, 17, 18, 18, **19,** 19, 19, **20,** 20, **21,** 21, 21, **22,** 22, 22, 22, 23, 23, **24,** 27, 28, 28, 28, 29, 30, 30, 30, 31, 31, 32, **34,** 34, 34, **35,** 37, **38,** 39, 44, **46,** 51, **52,** 53, **57,** 86, 107, S
	C.	50	18	1, 2, 2, 3, 4, 4, 4, 4, 7, 8, 8, 9, 10, 10, 11, 11, 12, 14, 14, 14, 15, 15, 17, 17, 18, 18, 20, 23, 24, 24, 25, 26, 28, 29, 29, 34, 34, 36, 36, 40, 40, 41, 42, 42, 102, 152, S, S, S, S
18	T.	50	27·5	**0, 6,** 8, **9,** 12, 14, **15,** 15, **16,** 17, 17, 18, 19, **20,** 20, **21, 21,** 22, **23,** 23, **24,** 26, 27, 27, 27, **28,** 28, **29, 30,** 30, 33, 33, 34, 34, **36,** 36, 37, **39,** 40, 40, **41,** 41, 42, 42, 46, 51, 54, 94, **S, S**
	C.	50	29·5	3, 4, 6, 8, 9, 9, 10, 14, 14, 16, 16, 17, 18, 18, 19, 20, 21, 22, 24, 25, 25, 25, 25, 28, 29, 30, 33, 34, 34, 34, 35, 37, 39, 40, 43, 51, 60, 80, 90, 111, 125, S, S, S, S, S, S, S, S
19	T.	50	21	0, 1, 1, 2, 2, **3,** 5, 5, 5, 7, **8,** 9, **10,** 13, 13, 13, 16, **17,** 17, **18, 18, 19,** 19, 20, **21,** 21, 21, 22, **23, 23, 23,** 23, **24, 24, 26,** 26, **27,** 28, 28, 28, 29, 29, 31, 31, 35, 40, **43,** 44, **51, S**
	C.	50	18·5	1, 2, 2, 2, 5, 6, 6, 6, 6, 7, 8, 9, 10, 10, 10, 11, 11, 11, 13, 13, 15, 16, 17, 18, 18, 19, 19, 20, 22, 22, 23, 23, 24, 24, 25, 25, 25, 29, 30, 32, 35, 36, 37, 40, 44, 45, 56, 57, 61, S
20	T.	50	18·5	**0,** 5, **6,** 6, 7, 8, **12,** 12, 12, **13,** 13, 14, **15, 15, 15, 15, 16,** 16, 16, **17,** 17, 17, 17, 17, 18, 19, 20, **21,** 21, 22, 22, **24,** 25, 26, 26, **29,** 30, 31, 31, 32, 32, 35, 36, 39, **41,** 41, **43, 70, 105, S**
	C.	50	18·5	0, 0, 1, 2, 3, 3, 3, 5, 7, 7, 8, 10, 11, 12, 12, 12, 12, 15, 15, 15, 17, 17, 17, 18, 18, 19, 19, 19, 20, 21, 22, 26, 28, 29, 30, 30, 34, 36, 36, 38, 40, 85, 91, 113, 138, S, S, S, S, S

T. trained line; C. control line. S indicates that the rat has had 'special training'. having failed to learn after 302 trials (and therefore with about 150 errors).

line, as should be the case if Lamarckian inheritance were operating. In nine generations the median of the trained line is higher, and in nine generations lower, than that of the corresponding control generations, and in the last generation the medians of the two lines are the same.

Table 3 brings out, however, the significant fact that there are periodic changes in facility of learning (as measured by number of errors made) which run roughly parallel in the trained and control lines. In the first four groups of generations there is a progressive decrease in the number of errors made by both lines, which is more or less stabilized in the fifth group (generations 17–20). Within each group there is also considerable fluctuation, showing a parallelism between the two lines, though, with the smaller numbers concerned, there is less regularity. In the last five generations, however, the parallelism is particularly close; the medians for generations 16–20 for trained and control lines are, in order of generations: 19 and 17, 19 and 18, 27·5 and 29·5, 21 and 18·5, 18·5 and 18·5.

Table 3. *Summary of the results of the first twenty generations in groups of four generations*

Generations	No. of rats		Median		% learnt with less than 10 shocks		% learnt after more than 100 shocks	
	T.	C.	T.	C.	T.	C.	T.	C.
1– 4	64	54	40·5	36·5	0·00	0·00	15·62	20·37
5– 8	116	91	34	29	5·17	9·89	13·79	6·59
9–12	105	177	28	27	9·52	7·91	4·76	7·91
13–16	187	148	17	22	26·20	23·65	4·81	3·38
17–20	230	200	21	21·5	18·26	20·50	3·04	12·00

T. trained line; C. control line.

These parallel changes in quickness of learning in the line with trained ancestry and the line without any trained ancestry is important for an interpretation of the positive Lamarckian effect claimed by McDougall. In fact, it may provide the clue to the discrepancy between his results and the generally negative results of Lamarckian experiments, including the result of Crew's test of McDougall's experiment. If, like McDougall, we had not maintained a control line, our experiment would have appeared to provide strong evidence, in spite of occasional irregularities, in favour of the theory that accumulation of trained ancestry progressively increases facility of learning in the descendants. Such an explanation is, however, invalidated by the fact that parallel changes in quickness of learning take place in the control line, without any trained ancestry. The cause of these changes is still obscure, but there are grounds for relating them to the general hygienic condition of our stock.

For a long period, from about generation 7–17, we were troubled with low fertility in our rat colony. Indeed, in some generations fertility was so low (only a small minority of rats producing any offspring at all) that we had to be content with many fewer rats in both lines than we wished. The extreme instance of this was generation 9, the trained line of which consisted of two litters only, from a single pair of rats. McDougall mentions that he also encountered periods of low fertility, and as his sixteenth generation consisted of five rats only, it is probable that it was descended from a single pair of parents. This low fertility in our stock was accompanied by obvious signs of bad condition, such as rough and scanty coats and the prevalence of 'rat pneumonia'. Finally, in generation 15 the colony became infested with mites, and in spite of efforts to keep the infestation in check, the experiment was in danger of coming to an end by the loss of the rats. Consequently, in generation 17 we decided to take radical measures to improve the condition of the stock. Up to that period, the rats had been kept in rather elaborate cages, with sleeping and feeding compartments and exercising turn-tables. For these we substituted a very simple type of metal cage which is easily sterilized by flaming. These cages were temporarily set up in another room, and before being transferred to them, every rat was anaesthetized and sponged with tincture of larkspur seed in 90% alcohol to destroy the mites, as recommended by Greenman & Duhring (1931). After all the rats had been transferred to the new cages, the original rat room was fumigated and the new cages returned. By this means the mites were entirely eradicated. The diet was also altered, chiefly by including more fresh milk and raw meat. An extraordinary improvement in both the general health and the fertility of the rats immediately followed this treatment.

McDougall and Crew have both expressed the view that weakly rats tend to learn more quickly than strong rats. In our first report we gave some statistical evidence supporting this conclusion; subsequent experience however has suggested that this factor is not very important. Nevertheless, this seemed at the time to give the explanation of the rise in the number of errors in generation 18, which coincided with the improvement in vigour of the colony. Moreover, this explanation seemed to be even more applicable to generation 17, the generation in which the change in housing and feeding the rats was made. In Table 2 the median of the eighty rats of the trained line is shown as 19. The generation can, however, be divided into two groups. The first forty-five rats were born and reared in the old mite-infested cages; the median of this group is 11. The median of the second group of thirty-five rats, born and reared under the new conditions, was 30. (In the control line of this generation all but six rats were born and bred under the old conditions.) The return to low training scores in generations 19 and 20, during which the rats continued in good condition, shows however that if there is a relation between health and rate of learning, it is not such a simple relation as generations 17 and 18 suggest.

A possible cause of fluctuation in rate of learning might be changes in the training procedure. There has been no change that we are aware of; indeed, the only factor in the procedure which appears likely to influence the number of errors made by the rats is the severity of the punishment for choosing the illuminated exit. So far as the severity is controllable, it depends upon the intensity and duration of the shock. These have been kept constant throughout the experiment by the methods described in our first report.

Selection may be suspected as a cause of a progressive change in the character of a population. We have already shown that, as judged by their training scores, the rats which became parents in the trained line were a representative group of the line as a whole. As we discussed in our first report, however, many accidental factors influence the number of errors made by a rat before it learns, and consequently differences in scores probably do not correspond closely with innate differences in learning ability, if indeed such exist among our rats. Consequently the fact that there was no selection of rats with high or low scores does not completely exclude the possibility that there may have been an accidental and concealed selection of potentially quick or slow learners, which, owing to the many chance factors influencing the number of errors made, did not reveal their true nature. If genotypic diversity is present in a population, and phenotypic diversity is not perfectly correlated with it, it is always possible that accidental genetic selection has taken place although there has been no phenotypic selection. It is therefore theoretically possible that the improvement both in the trained and control lines in the earlier generations was due to concealed selection of genetically quick learners. However, the fact that changes in rate of learning have occurred in both trained and control lines, and, moreover, that these run roughly parallel with each other, makes it very improbable that they have been caused by accidentally selecting for parentage genetically quick learners at some periods and of slow learners at others, which in the trained line did not reveal their true nature by their training scores. Moreover, McDougall obtained a progressive improvement in a line bred exclusively from the slowest learners in each generation, requiring on the selection hypothesis a series of coincidences between genetically quick and phenotypically slow learners too unlikely to be considered seriously.

For the present, therefore, it is not possible to state confidently the reason for the fluctuations in the rate of learning which have taken place during the course of the experiment. The all-important conclusion is, however, that since changes in rate of learning are roughly parallel in the trained and control lines, the progressive decrease in the number of errors over a long series of generations cannot be ascribed, in the trained line, to the cumulative effect of their trained ancestry.

THE PRESENT POSITION OF McDOUGALL'S 'LAMARCKIAN EXPERIMENT'

McDougall's fourth report brings the results of his experiment to generation 44. The part of the experiment on which conclusions as to an increasing facility in learning the task must be based begins, however, with generation 13, the first generation shown on McDougall's table. This is partly because in the first eight generations a different training procedure was used, which proved unsuitable; not till the 9th generation was his present procedure adopted, which is also essentially that followed by Crew and ourselves. Since training in his 10th, 11th and 12th generations was too incomplete to furnish data comparable to those from later generations, the figures available for comparison with Crew's and our own results are those for his generations 13–44, from which generation 20 is omitted as it was incompletely trained. These thirty-one generations show a marked, though naturally not quite regular, decrease in the average number of errors made by each generation, in the number of errors made by the worst rat, and still more in the number of errors made by the best rats, of each generation. The statistical significance of the decrease seems indubitable. Moreover, an independent experiment carried out in the same way, except that, in each generation, only the rats making the most errors were used as parents—thus introducing adverse selection—showed an even greater improvement than the main experiment. The criticism which must be levelled against McDougall's technique is that, although he trained four small batches of controls at different times, he failed to maintain a control line, so that it is impossible to know whether there was any change in learning facility taking place, which could not be attributed to ancestral training, as in our own control line.

The results of Crew's experiment provide very strong evidence that, where other factors tending to change the facility of learning the task are not operating, no improvement of learning facility takes place under the influence of increasing numbers of generations of trained ancestors. Crew's experiment was carried out for eighteen generations, and with a much larger number of rats per generation than used either by McDougall or ourselves. His experimental procedure differed in several respects from that of the other two experiments, notably in the fact that instead of giving six preliminary runs in the tank without shock, he gave a hundred, consisting of fifty runs with both gangways equally illuminated and no shock, followed by fifty with alternating light but no shock. This technique has made it possible for Crew to draw some interesting conclusions about the learning process, the formation of right and left habit, and so forth, about which, however, we are not here concerned. The important fact is that no evidence of increasing learning facility was obtained, either in the trained or control line. In neither line was there a progressive diminution in the mean or median number of errors, or in the scores of the best and worst rats, of each generation.

This is shown by Crew's Table 2. As, however, McDougall has stated that this table furnishes evidence 'not entirely without indications of improvement of facility in the course of the eighteen generations of training' (McDougall, 1938, p. 345), it is necessary to examine the strength of the three indications of improvement which McDougall mentions. He cites the fact that in the trained line the score of errors for the median rat of the last generation is the lowest of the series, and that the average error of the same generation is the lowest but one. When, however, the series of generations is considered as a whole, it is evident that Crew is fully justified in drawing the conclusion that there was no improvement. The eighteen generations arranged in order of descending medians are as follows: 9, 7 and 8, 6, 11, 17, 12, 10, 14, 1, 16, 15, 13, 5, 4, 2, 3, 18, and in order of descending average errors: 9, 11, 7, 8, 17, 14, 6, 12, 15, 10, 13, 16, 4, 1, 5, 2, 18, 3. McDougall's third indication, that an improvement of learning facility took place in Crew's trained line, is that twenty-nine rats in this line made no errors, and only ten in the control line. In view of the small number of these zero-error rats in both lines, this is a very slender indication, especially as the discrepancy is diminished by the fact that the total number of rats in the trained line was 1449 as against 1014 in the control line. McDougall continues: 'It would be of interest to know the distribution of the 29 errorless rats, and of others of small numbers of errors throughout the experimental series.' He seems to have overlooked the fact that this distribution is given in the pedigrees furnished by Crew. From these it can be found that the rats with zero scores do in fact occur more frequently in the later, than in the earlier generations. But this applies to the control line as well as to the trained line. In the latter, seven appear in the first nine generations, and twenty-two in the second nine, while in the control line three appear in the first nine, and seven in the second nine generations.

The suggestion that Crew's results, contrary to his own statement, do give indications of improvement of facility in mastering the task cannot therefore be substantiated. It must be accepted that Crew's experiment revealed no increasing facility in mastering the task, though he used much larger numbers of rats than McDougall did, and carried it through many more generations than was required by the latter to obtain this effect.*

McDougall, however, has also suggested two reasons why Crew's procedure may have been less likely to produce a Lamarckian effect than his own. The first is that for some reason Crew's rats were presented with a very much easier task than McDougall's. He bases this on the average number of shocks received by Crew's rats compared with the average for the various batches of rats of untrained ancestry trained by himself at various times. From this he draws the conclusion that Crew may have failed to obtain a Lamarckian effect, owing to the comparative ease with which his rats were able to learn their task, and the large number of them which received very few or even no shocks. He makes a similar criticism about our own experiment. In estimating whether the task provided by Crew and ourselves was, or was not, sufficient to produce a Lamarckian effect, the relevant comparison is not, however, solely between the number of shocks required by our rats to learn the task, and the number required by various control batches trained by McDougall. Equally relevant is a comparison between the number of shocks received by our rats, and the number received by McDougall's during that part of his experiment in which the Lamarckian factor was believed to be revealing itself, which includes the whole of the thirty-one generations of his main experiment (generations 13–44). During this period the number of rats which received no shocks was 29,* or 4·1 % of the total number of rats; in Crew's trained line the number was also 29, but owing to the larger total number of rats, this is only 2·0 % of the whole. If, therefore, the number of rats receiving no shocks is a reason for explaining the lack of a Lamarckian effect, it applies less to Crew's than to McDougall's experiment. As only eight of our 1372 rats have achieved the criterion of mastering the task with no shocks, this particular criticism does not apply to our experiment.

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* This must not be interpreted as implying that we accept Crew's explanation of the decreasing number of errors obtained by McDougall.

When we turn to the average number of shocks received, we find a similar weakness in McDougall's argument. The average number of shocks received by the 1445 rats of Crew's trained line is 43·4. Omitting McDougall's first generation (generation 13), where the average could not be given owing to some of the rats not having completed their training, we find that the mean number of shocks received by the 677 rats of his generations 14–44 is 33·3 (this figure is approximate only, as in all but one generation the average number of shocks is given in whole numbers).

Owing to our method of giving 'special training' to those of our rats which have not learnt after 302 trials (approximately 150 shocks) we cannot give an average strictly comparable with this. But even if we take 150 for the scores of the thirty-eight rats which received special training, thereby reducing the real mean by an unknown amount, the average number of shocks received by the 702 rats of our trained line is 33·9, as compared with McDougall's 33·3. Our experience with further training of rats which had exceeded 150 errors (our first report, p. 198), as well as extrapolation of the distribution of shocks in Table 1, shows, however, that even 200 is probably an underestimate of what the average score of these thirty-eight rats would have been, if their training had been completed in the usual way. Taking it as 200, the average number of shocks for the whole of our rats of the trained line is 36·6.

There is, therefore, no justification for explaining the failure to obtain the Lamarckian effect in Crew's and our own experiments on the grounds that our rats were presented with an easier task than McDougall's, if the basis of comparison is, not the isolated batches of controls trained by him, but the performance of his trained line during the whole period in which the Lamarckian effect was supposed to be revealing itself.

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* McDougall, however, does not count it an error when a rat swims up to the bright ramp, touches it with its nose, and on receiving the shock turns away, and swims out by the other passage. He says (1938, p. 329) that if these had been counted as errors (as we have done in our own experiment), then the number of cases of zero error would have been considerably reduced.

The second argument which McDougall has advanced, as a possible explanation of Crew's failure to produce any increase of facility in his trained line, is that Crew started the actual training of his rats to avoid the shock when they were 75 days old, whereas McDougall himself began it when they were about 30 days old. (This objection cannot be applied to our experiment, for we give our rats their preliminary six runs in the tank without light or shock when they are 27 days old, and start training proper on the following day.) He considers it a justifiable assumption that the earlier the age at which the training begins, the more likely is it that Lamarckian transmission of its effects may ensue. It is, of course, impossible to gauge the strength of this argument, but, if the assumption is justified, it greatly restricts the utility of the Lamarckian factor as an explanatory theory of the evolution of instincts connected with mating, nest-building, care of the young, migration, and all the other activities of organisms which are not brought into play until they are mature.

SUMMARY

This experiment, devised to test McDougall's claim that the effect of training in rats is inherited, has been carried out for twenty generations. In addition to the trained line, a control line has been maintained parallel with it, from which a number of rats have been trained in each generation, but not used for breeding. For each generation of the trained line there is therefore a corresponding group of trained control rats for comparison, differing from the rats of the trained line only in that they have no trained ancestry. During the first fifteen or sixteen generations there was a progressive, though irregular decline in the number of errors made in each generation in both lines. In generation 18 both lines showed a marked increase in the number of errors made, with fluctuations in subsequent generations running closely parallel in the two lines. This parallelism of periodic fluctuations in rate of learning in the two lines makes it impossible to attribute a progressive change in the trained line, when it happens to be in the direction of decreasing number of errors, to the inherited effects of ancestral training. Our experiment is being continued, and therefore our conclusions must be regarded as tentative only. The results of the experiment up to the present, together with those of Crew's experiment, show however, that the progressive decrease in the number of errors in successive generations of McDougall's experiment, in which no control line was maintained, cannot be held to have established the operation of Lamarckian inheritance.

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THE RELATION OF ANIMAL SIZE TO OXYGEN CONSUMPTION IN SOME FRESH-WATER TURBELLARIAN WORMS

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(With Two Text-figures)

INTRODUCTION

In the course of work started four years ago on the metabolism of fresh-water turbellarians from different habitats it became necessary to investigate the effect which body size had on the respiratory rate of the worms. The work had to be discontinued at the outbreak of war, but at that time the data concerning the effect of size on oxygen consumption were fairly complete and the chief results, which are given in this paper, may be of interest in the general subject of the effect of animal size on metabolism.

The size factor in the metabolism of animals is now recognized as one which must be taken into account in all metabolic studies. A considerable amount of work has been done in the past from the point of view of the size factor itself, and an extensive literature on the subject now exists. It would be out of place to attempt here to review the work which has been done; the homoiotherms which have largely figured in past investigations present different problems from those of the poikilothermal animals such as the turbellarian worms on which my work was done, and a summary of previous work on the poikilotherms has recently been given by Wingfield (1939). I will therefore confine myself to the Turbellaria. The work of Allen (1918), on *Planaria agilis* and *P. maculata*, and that of Hyman (1919), on *P. dorotocephala*, *P. velata* and *P. maculata*, demonstrated the higher respiration per unit weight of smaller individuals; the analysis of the relation between size and respiration was not taken further.

MATERIAL AND METHODS

In the work described below the oxygen consumption of four fresh-water species of Turbellaria was investigated.

Crenobia (*Planaria*) *alpina* (Dana) is particularly characteristic of cold springs. According to Beauchamp & Ullyott (1932) it is typical of rheocrene springs—that is, the type of spring in which the water begins to flow immediately from the source. This was the type of habitat in which I found the animals used in my experiments. I investigated the respiration of animals from two sources: during the summer of 1937 at the Freshwater Biological Station, Wray Castle (Westmorland), where the animals were collected from a spring at a temperature of 12–13° C.; during the summer of 1938 at the Biologisches Station at Lunz, where the prevailing temperature of the spring from which the animals were collected was 7–8° C. At Lunz the animals reached a much larger size than those at Wray, individual worms often exceeding a wet weight of 17 mg., whereas the largest worms at Wray rarely exceeded 6 mg. This is probably another case of the effect of environmental temperature on maximum size (Fox, 1939).

Polycelis cornuta (Johnson) is typical of cold brooks, tending also to replace *Crenobia alpina*, according to Beauchamp & Ullyott (1932), in springs of the limnocrene type where a stagnant pool is formed at the source. The animals used in these investigations were sent from Wray during the summer of 1939.

Polycelis nigra (Ehrenb.) is usually found in weedy ponds, but is also found to some extent on stones towards the edges of brooks. The animals used in my work were collected from a pond at Alvechurch, Worcestershire, in the spring and summer of 1939, during which time the diurnal temperature of the pond water fluctuated from 11·7 to 14·2° C.

Planaria polychroa (O. Schm.). I have observed this species to be present in weedy ponds only, but Whitehead (1921–2) summarizes its habitats as 'slowly running or stagnant water'. The animals used in my work came from a pond in Newdigate, Surrey,* and were collected during the spring and early summer of 1939.

Careful attention was paid to the treatment of the animals before their use in respiration experiments. All four species could be easily maintained in the laboratory, food being provided by pieces of *Gammarus pulex*. The animals were transferred immediately after collection to water artificially buffered with sodium bicarbonate so that it had the same alkali reserve as the water in which the animals were found in the field. This was considered necessary in view of the work of Anderson (1927) on the effect of alkali reserve on the oxygen consumption of *Planaria dorotocephala*. The respective alkali reserves were: *Crenobia alpina* 0·0004*N* at Wray, 0·0030 at Lunz; *Polycelis cornuta* 0·0010*N*; *P. nigra* 0·0025*N*; *Planaria polychroa* 0·0030*N*.

For the majority of experiments the oxygen consumption was determined at 14·5° C., and the animals were kept in the laboratory at this temperature. The animals were starved for 48–72 hr. before the experiments and were kept in dim light both before and during the experiments. The methods used in determining oxygen uptake were essentially those of Fox & Wingfield (1937, 1938). The animals to be tested were placed in glass stoppered respiration bottles which were completely filled with water artificially buffered to the appropriate alkali reserve. The oxygen content of the water in the bottles was determined at the beginning and end of a definite period of time by the method of Fox & Wingfield (1938). The respiration bottles were of 15 c.c. capacity for the smaller animals and of 75 c.c. capacity for the larger specimens, the number of animals introduced into each bottle varying with the size of the individual animal. A combination of size of bottle, size of individual animal and number of animals was chosen so that there should be a reduction of oxygen content of the water of 10–15% in 2 hr. The average number of animals used per experiment for each size group is given in Tables 1 and 2. The initial oxygen content of the water in the bottles was always approximately that of air saturation at 14·5° C., namely, 7·10 c.c. of oxygen per litre.

The experiments on *Crenobia alpina* at 7·0° C. were performed in a similar manner, except that the animals were kept at 7·0° C. before and during the experiments. The initial oxygen content of the water in the bottles in these experiments was approximately 8·0 c.c. of oxygen per litre.

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* The animals were supplied by Mr L. Haig, to whom thanks are due for despatching them to Birmingham.

The oxygen consumption was calculated per unit wet weight of animals. A standard method of weighing was used and its reliability tested by repeated weighing of the same batch of animals. The animals survived the weighing procedure if returned to water, but in the experiments given in this paper a particular batch of animals was used only once.

RESULTS

The results of the experiments at 14·5° C. are summarized in Table 1 and Fig. 1. Table 1 gives the number of experiments done on each size group in each species and the average number of animals used per experiment for each size group. The size range given refers to the limits of the average weights of the animals used in the experiments of each group.

Table 1. *Oxygen consumption of the different size groups of each species at 14·5° C.*

Species and locality	Size range mg. wet wt.	Average size mg. wet wt.	No. of exps.	Av. no. animals per exp.	Oxygen consumption cu.mm./g./hr.
Crenobia alpina (Wray Castle)	1·7– 3·0	2·3	19	75	186
	3·2– 4·1	3·6	16	47	175
	4·6– 5·9	5·4	11	42	148
Crenobia alpina (Lunz)	1·5– 3·9	2·8	6	17	178
	4·1– 7·1	5·5	14	22	141
	8·0–11·9	9·8	13	19	119
	12·6–17·0	14·0	17	16	119
Polycelis cornuta	1·6– 2·8	2·3	10	22	199
	3·1– 5·9	4·5	11	14	173
	6·3– 9·8	8·3	7	7	159
Polycelis nigra	1·5– 2·8	2·2	7	16	135
	3·1– 4·8	4·0	11	14	163
	5·0– 6·8	5·9	11	11	166
	7·2–10·8	8·7	9	6	176
Planaria polychroa	1·7– 4·7	3·5	11	15	116
	5·0– 6·3	5·8	9	11	110
	7·6– 9·7	8·5	9	10	98
	10·0–12·3	10·8	9	8	101
	13·1–14·7	14·2	7	5	94
	15·1–19·6	16·9	7	5	97

The mean oxygen consumption for the experiments on each size group is given in cu.mm./g./hr. The standard errors of the means are not given in the table, but they never exceeded 6·6 % of the mean oxygen consumption value. This indicates, I think, that the turbellarians used in these experiments were quite uniform from the metabolic viewpoint, since larger standard errors are more general in metabolic studies of this nature.

It will be seen that, with the exception of *Polycelis nigra*, the small animals of each species have a higher oxygen uptake per unit weight than the large ones. The graph relating size and respiration per unit weight for these three species is a curve of a hyperbolic or exponential form.

In the case of *P. nigra*, however, small animals have a lower respiratory rate than large ones. Since the standard errors of the means were in this case rather large compared with the differences between the means I thought it necessary to apply further statistical analysis to test the correlation between size and respiration. The data were considered as a case of curvilinear regression and the correlation ratio (r) worked out according to the procedure given by Peters & Van Voorhis (1935). The correlation ratio for respiration per unit weight (r) on individual weight (W) is 0·560 with a probable error of 0·075. Since the correlation ratio is more than seven times its probable error a definite correlation between r and W can be said to hold. The reason why *P. nigra* is different from the other three species is quite obscure and the matter requires further investigation.

Returning to a consideration of the other three species the question arises as to whether the size factor obeys the surface law of Rubner (1883). This well-known law, relating metabolism to size, postulates a constancy of the metabolic rate per unit body surface and would require in the present instance that the respiration per unit weight (r) should be related to the individual body weight (W) according to the formula $r = kW^{-\frac{1}{3}}$, k being a constant for a particular species under a particular set of conditions. Since Voit (1901) originally proposed that the surface law should hold for cold-blooded invertebrates as well as for the warm-blooded vertebrates with which Rubner was largely concerned, several investigators have demonstrated that it does in fact hold for many different animal phyla. The most important work of this nature on invertebrates which has come to my notice is that of Cohnheim (1901) on *Holothuria*; Hesse (1910) on *Helix*; Weinland (1918) on *Anodonta*; Hino (1929) on the earthworm *Pheretima*; Delpech & Terroine (1931) on *Helix*; Krüger (1940) on *Ascaris*. So far as the cold-blooded aquatic vertebrates are concerned there is only the work of Cronheim (1911) which shows the application of the

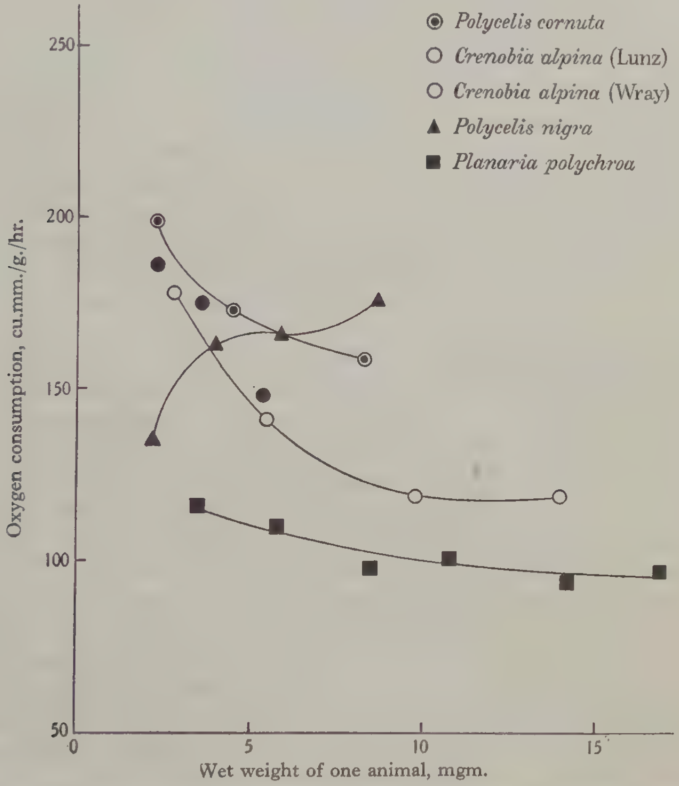

Fig. 1. Effect of individual size on the oxygen consumption per unit weight for all four species studied at 14·5° C. The points are plotted from the data given in Table 1.

surface law to the respiration of certain fishes, although I have found that the law can be demonstrated to hold also for the data given by Wells (1935) for the oxygen consumption of the fish *Fundulus*. Several other investigators have demonstrated the existence of a size factor in the metabolism of invertebrates but have not analysed their results from the point of view of the surface law. Most of these references are given in Wingfield (1939), but the work of Scherbakov (1935) on planktonic Crustacea is not mentioned.

In the present work it became apparent that the turbellarian worms are particularly suitable for the analysis of the size factor of oxygen consumption in terms of the surface law. Most of the previous work has depended on the application of the formula of Meeh (1879) for obtaining the surface area of an animal from its weight. Meeh stated that the surface area of an animal was proportional to the $\frac{2}{3}$ power of its weight, and the truth of this has several times been demonstrated, particularly for warm-blooded animals. The computation of the surface area of an invertebrate is often, however, a difficult matter owing to the irregularity of the surface and, very often too, to the existence of respiratory surfaces wholly or partly on the outside. This difficulty has led in the past to the attribution of different meanings to the 'surface' as found in Meeh's formula (Voit, 1901; Weinland, 1918; Pfaundler, 1921). Since, however, Turbellaria have a simple body surface and the proportions of the animals are largely maintained for different sizes of animals, Meeh's formula must hold with a considerable degree of accuracy.

Nevertheless, quite apart from the anomalous case of *Polycelis nigra*, it is obvious from Fig. 1 that the surface law does not always hold even for the other three species. *Planaria polychroa* has only a small size factor and the surface law is certainly not obeyed. The adherence to the law which is shown by *Polycelis cornuta* and *Crenobia alpina* at 14·5° C. can be seen from Fig. 2 (the upper two graphs) in which the logarithm of r (respiration per unit weight) has been plotted against the logarithm of W (individual weight). If the surface law holds, such a graph should be a straight line with a slope of $-\frac{1}{3}$. It will be seen from Fig. 2 that the graphs in the two cases under discussion do in fact approximate to straight lines with slopes of $-\frac{1}{3}$, particularly if the points for the largest *C. alpina* are disregarded. The slope for *C. alpina* works out at $-0{\cdot}34$, which is a good approximation to the theoretical value of $-0{\cdot}33$, so that it may be assumed that the surface law does hold in this case. For *Polycelis cornuta*, however, the slope is $-0{\cdot}18$, which is a considerable divergence from the theoretical requirement and it must be concluded that the respiration per unit surface area is not constant in *P. cornuta* but becomes progressively lower in smaller animals.

It is possible that there are other factors affecting metabolism which are determined by size of animal. In this case we may expect a divergence from the surface law which depends very largely on the existence of a direct relationship between size and metabolism, although no explanation of this relationship has been given which can be verified by further experiment. This latter problem has recently been discussed by Krüger (1940), but the whole subject seems to me to be very controversial and not profitable for discussion until more work, particularly that of a biochemical nature, has been done on the immediate reasons for the increased metabolism of small animals. However, it is of great interest to investigate other factors which may bring about the size relationship indirectly, particularly age and sexual maturity. I intended to investigate these in the Turbellaria, particularly as it seemed likely that the larger worms would be more sexually mature.

However, I have as yet no conclusive data on this point. Seasonal variations of metabolism, such as those noticed by Wells (1935) in *Fundulus* and by Morgan & Wilder (1936) in the mayfly nymph *Hexagenia*, could not have vitiated the results given above, since the work on each species was completed within a few weeks and an attempt was made also to include the whole size range in each series of experiments.

Comparatively little work has been done on the effect of other environmental conditions on the size factor in metabolism. The state of nutrition is almost certainly of importance and Allen (1919) has shown for *Planaria maculata* and *P. agilis* that the respiration per unit weight decreases on starvation after feeding until it reaches a constant level, although during the whole of this period the weight of the individual animal is gradually decreasing. In my investigations I aimed at achieving a normal and standard state of nutrition for the experimental animals.

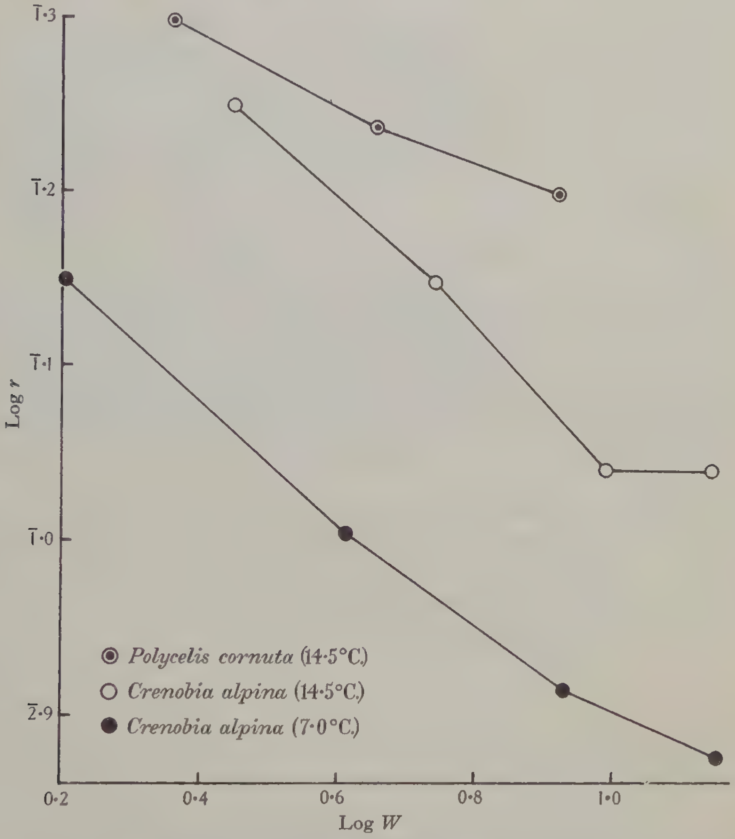

Fig. 2. Logarithms of *r* (respiration per unit weight) plotted against logarithms of *W* (individual weight). The values of *r* and *W* for *Polycelis cornuta* and *Crenobia alpina* (Lunz animals) at 14·5° C. are taken from Table 1. The values of *r* and *W* for *Crenobia alpina* (Lunz animals) at 7·0° C. are from Table 2.

Hotovy (1938), using the branchiopod crustacean *Triops* (*Apus*) *cancriformis*, has demonstrated the dependence of the size factor on temperature; it was diminished at lower temperatures. I have investigated the effect of temperature in the case of *Crenobia* *alpina*. The experiments were done at Lunz at 7° C. at about the same time as the 14·5° C. experiments, so that the two sets of results can be compared for the effect of temperature on the size factor. The results for different sizes at 7° C. are given in Table 2 and illustrated in Fig. 2 (lower graph). It is clear that, in the case of *C. alpina*, a decrease of temperature of 7·5° C. has only a slight diminishing effect on the size factor. The slope of the logarithm graph as obtained from Fig. 2 is −0·31, which is only slightly different from the 14·5° C. value of −0·34 and is still a good approximation to the −0·33 theoretically required by the surface law.

Table 2. *Oxygen consumption of* Crenobia alpina *at* 7·0° C. *Experiments done at Lunz*

Size range mg. wet wt.	Average size mg. wet wt.	No. of exps.	Av. no. animals per exp.	Oxygen consumption cu.mm./g./hr.
0·9– 2·3	1·6	7	28	141
3·1– 5·1	4·1	7	31	101
6·5–11·1	8·5	7	22	82
12·4–17·4	14·3	8	16	75

The place of origin of the experimental animals has no apparent effect on the nature of the size factor. This is well brought out in a comparison of the results obtained for *C. alpina* at Wray and Lunz. Fig. 1 shows that there is good agreement between the two sets of results, and this is despite the fact that the prevailing environmental temperatures were different at the two places (12–13° C. at Wray, 7–8° C. at Lunz), and that the size ranges at the two places were 1·7–5·9 mg. per animal at Wray and 1·5–17·0 mg. per animal at Lunz.

With regard to the magnitude of metabolic rate for the different species it will be noticed that for the species which show a normal size factor (i.e. small animals having a higher metabolic rate than large ones) the stream species have a higher respiratory rate than the pond species. This is in agreement with the findings of Fox *et al.* (1933, 1935, 1937) for other fresh-water poikilotherms.

SUMMARY

1. A size factor is demonstrated for the oxygen consumption at 14·5° C. of four species of fresh-water Turbellaria obtained from different habitats.

2. For three of the species studied (*Polycelis cornuta*, *Crenobia alpina* and *Planaria polychroa*) small animals were found to have a higher oxygen consumption per unit weight than large ones. In the case of *Polycelis nigra* small animals were shown to have a lower oxygen consumption per unit weight than large ones.

3. The adherence of the size factor to the surface law of Rubner is discussed. Only *Crenobia alpina* satisfies the theoretical requirements of the law, and in this species it is shown that the size factor is practically unchanged when the oxygen consumptions are determined at 7·0° C. instead of 14·5° C.

I am very grateful to Prof. H. Munro Fox, F.R.S., of Bedford College, London, for the constant encouragement he gave me during the course of the work and for the valuable help given during its publication.

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STUDIES ON THE PHYSIOLOGY OF *ARENICOLA MARINA L.*

III. THE POTASSIUM RELATIONS OF THE ISOLATED EXTROVERT (WITH REMARKS ON THE EFFECTS OF VARYING THE LEVER WEIGHT)

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(*Received 4 July 1942*)

(With Six Text-figures)

The 'isolated extrovert' of the lugworm consists of the proboscis with a short length of oesophagus attached. It remains active for over 24 hr. in sea water and gives a remarkable rhythmic pattern consisting of outbursts of vigorous activity alternating with periods of relative rest (Wells, 1937). We have already published its reactions to changes in osmotic pressure and magnesium concentration (Wells & Ledingham, 1940*a*, *b*). We now continue our study of its relations with its bathing medium by describing the effects of varying the potassium concentration. As many authors have found that potassium: magnesium antagonism is important in invertebrate preparations, and as the extrovert behaves normally in a fluid whose magnesium content is high, we have paid particular attention to the possibility of antagonism between the two ions in the present case.

METHODS

The work was done at Bangor, North Wales. The movements of the extroverts were recorded with light isotonic levers, exerting a pull of 0·35 g. on the preparation, except where otherwise stated. The extroverts were mounted in a cylindrical jar of fluid, which was changed by completely siphoning off the old and pouring in new; this operation takes about a minute and causes an upward movement of the lever at the time of change, which is *not* due to contraction of the preparation.

Salines were made by mixing solutions each isotonic with sea water. Artificial sea water, which was taken as the 'normal' starting point, was made up as follows:

Potassium chloride, 0·6 *M*, 1·8 c.c.
Magnesium chloride, 0·4 *M*, 14·5 c.c.
Calcium chloride, 0·4 *M*, 2·8 c.c.
Sodium sulphate, 0·55 *M*, 5·5 c.c.
Sodium chloride, 0·6 *M* (to 100 c.c.)

After the extrovert had settled down in this mixture, it was exposed to another, in which the amount of potassium, or of potassium and magnesium, chlorides were abnormal; then, after an exposure usually of several hours, the effects of return to artificial sea water were recorded.

It will be seen, as all mixtures are finally made up to 100 with NaCl, that variations in KCl or $MgCl_2$ are osmotically compensated by reciprocal variations in the amount of NaCl included. Osmotic pressure, Ca concentration and SO_4 concentration are therefore constant. All solutions were made up in Na bicarbonate $N/400$ and were thoroughly aerated before use; this kept the *p*H constant at 8·0–8·2. Experiments were made at temperatures of 11–21° C.; over this range the preparations behave regularly and well. The temperature did not change significantly in the course of any one experiment.

RESULTS

Concentrations of K and Mg are given below as multiples of their concentrations in the artificial sea water; thus K 1 is 0·0108 *M* and Mg 1 is 0·058 *M*. We find that there are four physiologically distinct ranges of potassium concentration, which we designate respectively as moderate excess, severe excess, moderate deficit and severe deficit. This diversity of potassium effects is due, as will be shown below, to the complexity of the normal behaviour pattern of the preparation. The effects in each range will be described separately.

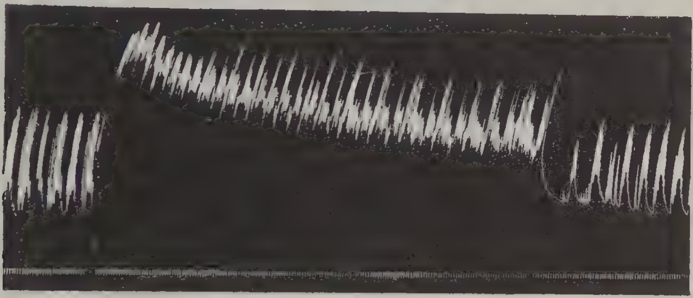

Fig. 1. Moderate K excess. Exposure to K 3, lasting 3 hr. 6 min. Read from left to right. Upstroke of lever means contraction of preparation. Time signal marks minutes. In all records (except Fig. 6), there are two breaks in the signal line, which show the times at which the fluids were changed. In each record the experiment begins and ends in artificial sea water, and, between the breaks, the extrovert is exposed to the fluid named in the legend.

Moderate excess

On changing from artificial sea water to K 2, 3 or 3·5, the preparation is at first excited; its tone rises and its activity becomes continuous. As the exposure continues, the extrovert accommodates itself to a large extent to the new medium, and the effects become less marked. On return to K 1 there is generally a clear phase of depression (subnormal tone and activity) which passes off as the preparation accommodates itself back again to the original medium (Fig. 1).

The whole picture is extraordinarily like the effect of diminishing the Mg at constant K concentration (Wells & Ledingham, 1940*b*), for in that case also one gets excitation and accommodation, followed by depression and accommodation on changing back to artificial sea water. It is therefore hardly surprising to find that the effects of moderate K excess can be fully antagonized if the Mg concentration is simultaneously raised. To demonstrate this antagonism, however, the two ions should not be increased in the same geometrical proportion. Change from artificial sea water to K 3 Mg 3 gives a slight Mg excess picture; the best antagonism is seen with K 3 Mg 2·0–2·2 (the exact ratio seems to vary slightly in different preparations). With such a solution, the effects of the two excesses cancel out completely and the preparation behaves as it does in artificial sea water (Fig. 2). Immediately after the change there may be a period of disturbance, lasting for up to 20 min., during which the effects observed resemble neither those of K nor those of Mg; or this stage may be absent, as in the figure, the preparation continuing its activity without even temporary alteration. In either case, the final result is the same.

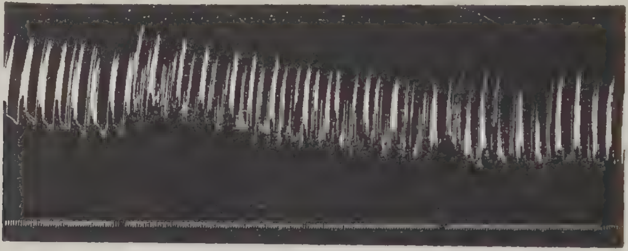

Fig. 2. K : Mg antagonism. Exposure to K 3 Mg 2, lasting 2 hr. 13 min.

Very slight excess (K 1·33 or 1·5) gives a small increase in rhythmic activity followed by accommodation, but no evident tone rise; the picture is like that shown in very slight Mg deficiency.

Severe excess

On changing from artificial sea water to K 5–10, there is contracture and inhibition of rhythmic activity (Fig. 3). The contracture can be regarded as an exaggeration of the tone rise seen with moderate K excess, particularly as it is greatest at the beginning of the exposure. The inhibition of rhythmic activity, on the other hand, could not be anticipated from a consideration of the effects of moderate excess, and shows that a qualitatively distinct concentration range has been reached. With K 5 there is very slight continuous activity of low amplitude, and without the usual intermittent pattern; with K 7·5 or 10

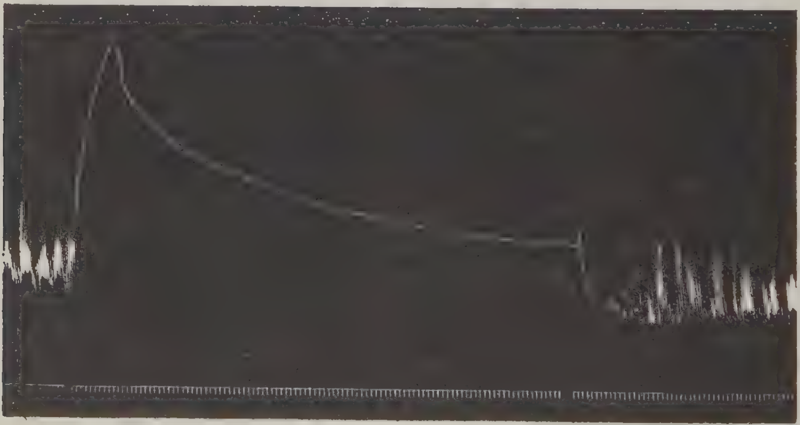

Fig. 3. Severe K excess. Exposure to K 7·5, lasting 1 hr. 40 min.

there is no rhythmic activity at all. In neither case can any tendency to accommodation, as evidenced by improvement in the rhythm, be discerned, even in an exposure of over 11½ hr.

On returning from artificial sea water, there is a sharp drop of tone and, if rhythmical activity was still visible under the conditions of K excess, it now ceases. After a period of complete quiescence, gradual recovery occurs.

The effects of great K excess are not substantially modified by simultaneous increase in Mg (e.g. change to K 5 Mg 5 or K 5 Mg 3). Contracture still occurs, although it is somewhat less marked than when Mg is held constant. The rhythmical activity, instead of being improved, is still further inhibited; this is the inhibitory effect of Mg excess added on to the effects of great K excess. Evidently, then, we are here concerned with phenomena of a different mechanism to those seen with moderate K excess. As regards rhythm, at least, the absolute concentrations of the two ions are too high to allow activity, even when they are balanced.

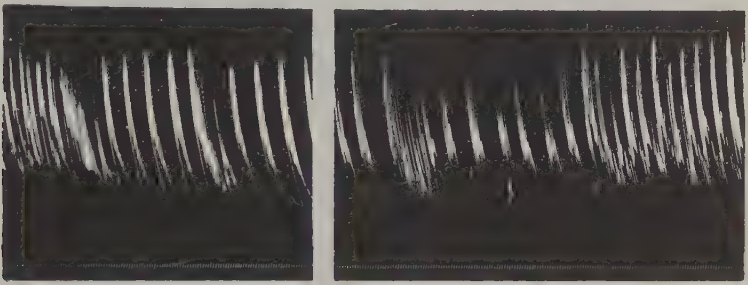

Fig. 4. Moderate K deficit. Beginning and end of an exposure to K 0·5, lasting 8 hr. 30 min.

The boundary between moderate and great excess lies in the neighbourhood of K 4. With this concentration there is rhythmic activity of considerably reduced amplitude in which indications of the normal intermittent pattern can nevertheless be distinguished; the latter is seen more distinctly if Mg is simultaneously raised to Mg 3.

Moderate deficit

Change from artificial sea water to any low K concentration from K 0·75 to 0·25 has the following results: (1) a sharp and persistent tone drop, (2) at first, a period of continuous rhythmic activity, which lasts the longer, the lower is the new K concentration, and (3) a modified form of the normal behaviour pattern then appears, in which the outbursts are separated by unusually long and unusually quiet rest periods, and every now and again an outburst of unusually long duration appears (Fig. 4). These modifications persist for many hours, with no sign of improvement or accommodation.

In some respects, the moderate K deficit picture recalls that given by Mg excess at constant K concentration (Wells & Ledingham, 1940*b*); in both cases there is a tone drop, a wider spacing of the outbursts, and a depression of activity between the outbursts. On the other hand, the initial excitement following the downward change, the occasional prolonged outbursts, and the absence of any sign of accommodation, differentiate the moderate K deficit picture from that of Mg excess. The result of simultaneously reducing

K and Mg was tried in a considerable number of experiments, with various combinations of concentrations varying from K 0·5 to 0·25, and from Mg 0·75 to 0·25. Full antagonism was never observed. By itself, a drop in Mg concentration has effects like those of moderate K excess; it causes tone rise and raised rhythmical activity, but does not bring the outbursts closer together. The general result of simultaneously decreasing K and Mg is the sum of the effects of decreasing each of the two ions separately; thus in respect of tone level and the amount of activity between outbursts, which are affected in opposite senses by the two ions, there is antagonism between them, whereas the wide spacing of the outbursts and the occurrence of occasional abnormally prolonged outbursts are shown under conditions of moderate K deficit, whether or not there is also a Mg decrease. As regards this particular preparation, K : Mg antagonism seems to be a factor of very limited importance, being complete only in the range of moderate K excess.

Severe deficit

On changing from artificial sea water to K 0·05 or 0·00, there is great excitement with partial contracture and continuous activity. The tone level then drops very slowly; the activity continues in a chaotic manner, without any sign of the normal behaviour pattern, for over 12 hr.; during this time there is a steady loss of amplitude. It is remarkable that this activity continues, though abnormal in pattern, for so long a time, even if the K-free bathing medium is repeatedly changed.

The reactions of the extrovert to severe K deficit in no way resemble those to Mg excess, and it is therefore hardly to be expected that they would be antagonized if Mg were simultaneously reduced. In fact, the simultaneous omission of K and Mg from the bathing fluid gives a record showing the sum of the effects of the two deficits. If Mg alone is suddenly withdrawn, there is contracture followed by a considerable degree of accommodation with restoration of the rhythm (Wells & Ledingham, 1940*b*). If both ions are withdrawn, contracture is still seen; the rhythm, instead of becoming fairly normal again, shows the disturbances resulting from severe K lack.

The potassium paradox

The effect of changing back from a K-deficient to a normal fluid depends partly on the extent of the deficit, and partly, in certain cases, on the time for which the tissues have been exposed to it. In a slight deficit, the extrovert settles down almost immediately into a condition in which the normal behaviour pattern is present, though modified. On changing back to artificial sea water, the original pattern is promptly resumed, without any noticeable temporary effects of the change (Fig. 4). In very severe deficit, the pattern disappears altogether, although irregular activity persists for some time; on changing back, there is a period of complete relaxation, after which activity is abruptly resumed (Fig. 5). This pause is the well-known 'potassium paradox', which has been observed on return from K lack in a great variety of rhythmic preparations (Wells, 1942). The borderline between moderate and severe deficit is, however, not at all clearly defined. On changing from K 1 to 0·25 or 0·20, there is generally an initial period of confused, continuous activity, superposed on a tone rise, and later the intermittent pattern, as modified by moderate K deficit, gradually emerges. The first stage resembles the beginning of an exposure to severe deficit, and the whole picture suggests that an accommodation process is taking place. The response of different preparations varies very greatly, especially in the duration of the first stage; this is sometimes well marked even with K 0·33. The suggestion that some sort of accommodation process occurs is confirmed by a curious fact; the K paradox appears on returning to sea water if the exposure was short, but not if it was long, e.g. it is shown after 1 hr., but not after 8 hr., in K 0·25. With severe deficit, on the other hand, there is no evidence of accommodation; the intermittent pattern shows no sign of returning, and the paradox is as well marked after 8 hr. exposure as after 1 hr.

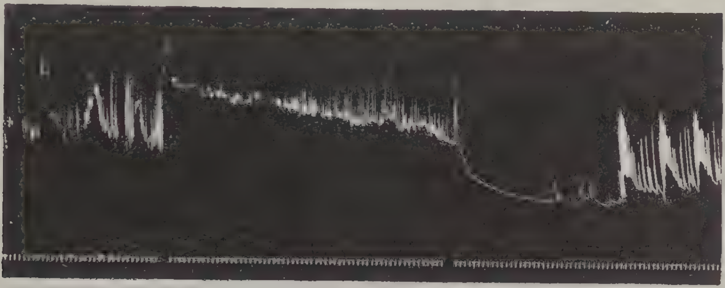

Fig. 5. Severe K deficit. Exposure to K 0, lasting 1 hr. 2 min.

DISCUSSION

A comparative account of the action of K on rhythmic muscles was recently published by one of us (Wells, 1942). On the whole, the *Arenicola* extrovert fits in pretty well with the general scheme. The peculiar feature of the extrovert is, of course, the elaboration of its performance; superposed on the simple rhythm of alternating contraction and relaxation, it shows a 'pattern' of alternating phases of vigorous rhythmicity and comparative quiescence. If for the moment we disregard the pattern, we find that the extrovert agrees in its general K relations with a great range of preparations of diverse functional and histological types; for with severe excess it shows contracture and inhibition of the rhythm, with severe deficit it shows a lasting tone rise and a rhythm which persists for some time, and on return to a fully balanced mixture from severe deficit it gives the 'potassium paradox'. The pattern is, however, very sensitive, especially to K lack. In the absence of K from the external medium, a snail or crab or frog heart will trace for some time a record which does not differ very greatly from that seen in a fully balanced fluid; with the lugworm extrovert, on the other hand, the pattern disappears altogether from the moment when K is withdrawn (although rhythm continues for many hours), and even slight K deficit is enough to make striking changes in the timing of the outbursts. There is a remarkable similarity between the outbursts of the extrovert and the 'grouped beats' which regularly beating muscles, such as hearts, sometimes show under abnormal conditions, and it has been suggested that the pattern represents the stabilization of a condition which occasionally appears in other preparations as a freak (Wells, 1937). If this be true, one would expect the pattern to be more sensitive to environmental factors than the basal features of rhythm and tone upon which it is superposed, and one finds in fact that it only appears in those K concentration ranges which we have designated as moderate. Under conditions of severe excess or deficit, the extrovert loses its distinctive intermittence and behaves like any one of a wide range of rhythmic muscles.

With regard to the mechanism of the intermittent rhythm, three points arise for discussion:

(1) From the available data, it is possible to draw certain conclusions about the dependence of the pattern on ion balances. The K concentration range compatible with its appearance falls into two sharply contrasting parts, for the effects of moderate excess are quite different from those of moderate deficit. In moderate excess, the disturbances resemble those produced by Mg lack, and can be completely abolished by an increase in Mg; the extrovert shows considerable powers of accommodation. We seem, then, to have a very perfect case of K : Mg antagonism. In moderate deficit (except for the special case of the borderline concentrations, K 0·25 and 0·20) there is no accommodation; the disturbances are in many respects unlike those produced by Mg increase and cannot be completely antagonized by lowering the amount of Mg; the balance between the two ions is therefore not the only, or even the main, factor. It would, however, appear that the effects of moderate K deficit are due to disturbance of a balance between K and some other, as yet unidentified, constituent of the medium. We showed elsewhere (Wells & Ledingham, 1940*a*) that the extrovert behaves with great regularity in sea water diluted to as much as four or five times its volume with buffered distilled water. As the total concentration of salts falls, the amplitude of the contractions becomes less, but the pattern remains apparently perfectly normal. If, therefore, all the ions are decreased together, the pattern is not disturbed; if, on the other hand, K alone is decreased, the characteristic distortions described above are seen. Clearly, then, K is working against some antagonist other than Mg. The identity of this antagonist is not yet known; perhaps the problem will be solved by further experimentation.

(2) The results obtained with moderate K excess enable us to develop further the discussion in our paper on Mg actions (Wells & Ledingham, 1940*b*), of the mechanism of accommodation to that ion. The main facts were: (*a*) a raised Mg concentration depresses, and a lowered one excites, the extrovert, (*b*) the extrovert slowly accommodates itself to a large extent to a new concentration, (*c*) after accommodation, return to artificial sea water causes excitement after raised Mg and depression after lowered Mg, and (*d*) the extrovert then slowly accommodates itself back into the original condition. To these we may now add: (*e*) the response to moderate K excess is almost exactly like that to lowered Mg, and (*f*) if K and Mg are simultaneously raised, the extrovert may show no significant response at all, either temporary or permanent.

The mechanism of the response to change in Mg concentration can be envisaged in various ways. The accommodation process might directly involve the Mg ion; for instance, the performance of the preparation might depend in some way on the relative amounts of Mg inside and outside the cells; a change in external Mg concentration would upset the balance, and Mg might then move slowly across the cell membrane to restore it. On the other hand, the disturbance caused by change in Mg concentration might be corrected by processes in which the ion itself is not involved. The strict antagonism observed between K and Mg makes the former suggestion hard to entertain. To fit the new facts in, awkward additional postulates would have to be made. It therefore seems to us that a hypothesis of the second type, in which the ions do not take part in the accommodation process, is simpler and more satisfactory.

Burton (1939) showed that accommodation is a general property of steady state systems under certain conditions. In our Mg paper (1940*b*), we developed a steady state model, to illustrate a possible mechanism of accommodation to that ion. The responses to moderate K excess can be admitted without difficulty into such a scheme. Consider, for example, the simple chain:

$$S \xleftrightarrow{k_0} A \xrightarrow{k} B,$$

where S is a source, of constant concentration, from which A is continually supplied by a reversible or irreversible process of velocity constant k_0; B is a device for measuring the rate of the irreversible reaction $A \rightarrow B$ (velocity constant k)—such, for example, as a relaxation oscillator whose frequency of discharge is proportional to the rate of inflow from A; and A is a variable concentration whose magnitude depends on the rate of supply from S and the rate of loss to B. Suppose that, when a steady state is reached and the rates of supply and loss are equal, some external factor causes k to change abruptly to a lower value. The rate of $A \rightarrow B$ will fall at once; therefore the concentration A will tend to rise; as it does so, the rate of $A \rightarrow B$ will rise again (accommodation) until a new steady state is reached. Similarly, a sudden increase in k will cause the rate of $A \rightarrow B$ to show a sudden rise followed by gradual accommodation. This was illustrated by means of a simple water model, which traced kymograph records imitating those of the extrovert (Wells & Ledingham, 1940*b*).

The extrovert itself, being alive, must necessarily contain steady state chains. If we assume that Mg and K act antagonistically on a velocity constant, under conditions comparable to those of the simple system just described, the observed facts receive a ready explanation.

Note that, if the supply reaction is irreversible ($S \rightarrow A$), its rate will be constant and will determine that of the whole chain when a steady state is reached—in other words, accommodation to a change in k will be complete. If it is reversible, the concentration A will affect the supply rate and accommodation will be partial. The latter condition is therefore suggested in the case of the extrovert.

(3) As with most rhythmic muscles, the performance of the *Arenicola* extrovert is affected to some extent by the pull of the writing lever, and the results obtained by varying that factor in the present case are similar in some respects to those got by varying the K or Mg concentration. Fig. 6 shows an extract from one of a series of experiments, in which the tension on the preparation was changed from 0·5 to 0·15 g. and back again, sea water being the bathing medium throughout. It will be noted that, at the higher tension, the extrovert (*a*) stretches considerably, (*b*) shows more activity in the rest periods between outbursts, and (*c*) shows a greater number of strokes, and a less conspicuous tone rise, in each outburst. The number of outbursts in unit time is not significantly affected.

The typical alternation of activity and rest periods is seen even in untied preparations lying in sea water. The fundamental pattern, therefore, is independent of tension, although the details can be considerably modified by varying that factor.

The effects of increasing the tension are very like those seen when K is moderately raised, or Mg moderately decreased (compare Figs. 1 and 6), and it might be thought that the two ions act, over the range in which they antagonize each other, by affecting the sensitivity of the extrovert to the pull of the lever. There is, however, an important difference between the response to a moderate change in the K : Mg balance and that to a change in lever weight. In the latter case there is apparently no accommodation. It therefore appears that the two factors, mechanical and chemical, produce similar end-results but in different ways.

Evidently, the working of a steady state system such as the one just described could be disturbed at various points, and the observed result of the chain could be made to change without showing accommodation. If the value of k_0 were suddenly to alter, the rate of $A \rightarrow B$ would move exponentially to a new value, without any temporary overshoot. If the sensitivity of B were to change (e.g. an alteration of threshold, in the case of a relaxation oscillator), the observed result of the chain would rapidly or immediately assume a new, final value. The effects of a change of tension might be produced in the latter way; the end-result would then be the same as that of a change in the K : Mg balance, but there would be no overshoot and accommodation. At present, this scheme is no more than a plausible explanation of the facts; it does, however, show that mechanical and chemical factors could operate in entirely different ways, even though the final picture is much the same in both cases.

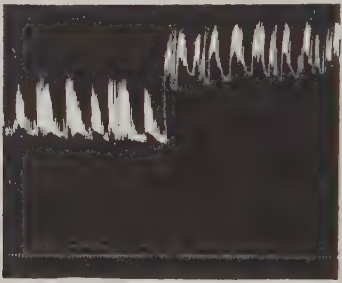

Fig. 6. Effect of tension. Preparation mounted in sea water. At first the tension on the preparation is 0·5 g.; this is changed to 0·015 g. at the break in the signal line.

It must be emphasized that the above remarks apply only to K changes within the moderate excess range. The contracture and inhibition produced by great K excess, and the effects of K deficit, are quite different from the results of varying either the Mg concentration or the lever weight.

SUMMARY

1. The effects on the isolated extrovert of *Arenicola marina* L. of varying the potassium concentration of the bathing medium are described. Data are also presented on K : Mg antagonism. Potassium and magnesium concentrations are given as multiples of their concentrations in artificial sea water, which was taken as the 'normal' starting fluid.

2. The extrovert normally shows a distinctive pattern of alternating periods of activity and rest, superposed on the more general properties of rhythm and tone. This pattern is very sensitive to changes in potassium concentration. Moderate changes produce modifications of the pattern. Severe changes abolish the pattern and produce effects on rhythm and tone resembling those shown by most rhythmic muscles under like conditions.

3. Moderate K excess (K 1·5–3·5) excites tone and rhythm. Accommodation occurs during long exposure. The effects resemble those of Mg deficit, and can be completely abolished by increasing the Mg concentration.

4. An increase of lever weight has effects on the rhythm resembling those of a moderate K excess or a moderate Mg deficit, but in this case there is no accommodation. It is suggested that the extrovert contains a steady state system such as

$$S \xleftrightarrow{k_0} A \xrightarrow{k} B$$

where S is a source and the performance of the extrovert depends, through B, on the rate of the process $A \rightarrow B$. If change in the K : Mg balance acts on k, its result will be following automatically by accommodation. Change in tension can produce the same end-result, without accommodation, by acting on B.

5. Severe K excess (K 5–10) causes contracture and inhibition of the rhythm. The contracture is partly antagonized by simultaneous increase in Mg, but the inhibition is not antagonized.

6. Moderate K deficit (K 0·75–0·33) causes initial excitement, then a characteristically modified pattern, with widely spaced activity outbursts, and an occasional abnormally long outburst. These effects are not antagonized by simultaneous decrease in Mg. As, however, they are not seen in preparations exposed to sea water diluted to four times its volume, they are due to disturbance of a balance between K and some other constituent of the medium.

7. Severe K deficit (K 0·05 or 0·00) causes partial contracture with chaotic activity, which lasts for many hours. Neither effect can be antagonized by simultaneous decrease in Mg. With borderline deficits (K 0·25 or 0·20) the preparation reacts at first as if to severe deficit, then accommodates itself and gives the pattern characteristic of moderate deficit.

8. The potassium paradox occurs on returning to artificial sea water after severe deficit. With borderline deficits (K 0·25 or 0·20) it is seen after short exposure, but not after long.

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THE FLUCTUATION OF *p*H AND ORGANIC ACIDS IN THE RUMEN OF THE SHEEP

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(With Two Text-figures)

It has long been known that bacterial fermentation of cellulose in the rumen is associated with the production of a mixture of lower fatty acids, methane and carbon dioxide. It was first shown by Pringsheim (1912) and later confirmed by Woodman & Stewart (1928) that the addition of toluene to thermophilic cultures of cellulose-splitting bacteria resulted in the accumulation of glucose, and Woodman (1930) suggested that glucose was the product that was of nutritional value to the animal. Broadly speaking, theories regarding digestion of cellulose fall into two categories; those that emphasize the importance of the chemical products of fermentation, glucose and the lower fatty acids, and those that regard the food eaten by the animal as a substrate upon which the organisms of the rumen thrive, the bacteria and Protozoa forming the end-product which is subsequently digested by the sheep. Pochon (1935, 1938) put forward an ingenious explanation which belongs to the first school of thought; he isolated a cellulose-splitting organism which he named *Plectridium cellulolyticum* from the rumen of the ox and found that if the *p*H of the medium in which active cellulose fermentation was proceeding was reduced to 4, glucose accumulated. This change is similar to that which occurs when ingesta pass from the rumen to the abomasum, and he suggests that glucose is formed in the abomasum of the animal and the lower fatty acids that are formed in the rumen are waste products. Baker (1939) supports the second theory. He studied the decomposition of plant tissue microscopically with the use of polarized light and was able to observe the breakdown of cellulose by the loss of double refraction in the cell walls. Bacteria were intimately related to the areas where erosion was occurring and these organisms were iodophilic. He considers that the bacteria besides exerting cytoclastic activity take up the sugars formed by hydrolysis of cellulose and so protect them from further degradation. These sugars are condensed to an amylodextrin type of polysaccharide and so produce the blue staining reaction with iodine. The bacteria are subsequently digested by the ruminant either before or after ingestion by the ciliates of the rumen.

It is not the purpose of this paper to discuss the relative merits of these schools of thought as both are correct in that large quantities of organic acids are produced in the rumen, and that the organisms of the rumen live and multiply upon the food the animal eats. The problem is to find out how much of the carbohydrate of the food, especially of cellulose, is conveyed to the animal as organic acid and how much is converted into bacteria and Protozoa or is stored by these organisms in one form or another. The value of these organisms as an end-product of fermentation must remain a matter of speculation until definite proof is forthcoming whether or not they are digested by the animal after they leave the rumen.

The data presented in this paper is the result of routine observations in which the fluctuation in the quantity of organic acids present in the rumen together with the consequent variation in *p*H of the ingesta was determined after feeding various rations. The results show that the lower volatile fatty acids are present in considerable quantity and must be considered to be an important end product of carbohydrate fermentation.

METHODS

The majority of experiments were done on four sheep, each provided with a rumen fistula; two were Dorset Horn ewes and two were cross-bred wethers. In addition, seven other animals were used; these included Hampshire Down and Ryland ewes besides the breeds mentioned. One of the Ryland ewes was operated upon when pregnant, and four months later she bore and successfully reared twin lambs. Two of the Hampshire Down ewes were provided with both rumen and abomasal fistulae.

The operative technique was that described by Phillipson & Innes (1939), except that in most cases the rumen cannula was brought out through a stab wound in a similar manner to that described for abomasal fistulae.

Samples of rumen ingesta were taken by suction through the rumen cannula, a trap consisting of a small filter flask being introduced between the tube entering the rumen and that to which suction was applied. The liquid ingesta thus obtained were filtered through muslin and all estimations are expressed as percentages of this fluid.

Volatile acids were distilled after acidification by the method described by Dyer (1916); frothing was troublesome when the crude liquid was used and protein was precipitated by the copper-lime method. Distillates obtained from the crude liquid and from the copper-lime filtrate from the same samples showed that no loss of volatile acid occurred during this treatment, in fact the filtrate gave slightly higher results than the crude liquid. Lactate was estimated by the method of Friedmann and his co-workers (1927, 1929), using the copper-lime filtrate. The hydrogen-ion concentration was measured electrometrically, using the quinhydrone electrode. Samples obtained from the rumen by suction gave high readings, and a special method of sampling was adopted. A small-bore tube with the thumb placed over the top was thrust well down into the rumen contents; on releasing the thumb, liquid rose up the tube and could be lifted from the rumen when the thumb was replaced again. The liquid was run into a test-tube containing quinhydrone and immediately covered with liquid paraffin; if the last operation was omitted the readings were not stable and slowly increased.

RESULTS

Preliminary experiments on two sheep showed that the *p*H of the rumen ingesta, although relatively constant, fluctuated in a definite manner throughout the day. The animals were kept on a ration of bran and oats mixed with chaffed hay and straw; the whole ration was given at 8 a.m. in the morning, the sheep fed during the first part of the morning and then again in the evening. The *p*H fell during the morning and reached its lowest level at midday, sometimes earlier; it remained at a low level during the afternoon or commenced to rise slowly. If the latter was the case, it fell after the evening feed, but if it had remained low, then no further fall occurred. The following figures are typical of the results obtained:

24 September 1939		4 October 1939	
Time	pH	Time	pH
9.45 a.m.	6·47	7.20 a.m.	6·70
10.45 a.m.	6·25	8.20 a.m.	6·63
11.45 a.m.	6·27	10.30 a.m.	5·69
1.00 p.m.	6·16	11.30 a.m.	5·68
2.30 p.m.	6·18	12.30 p.m.	5·98
3.30 p.m.	6·43	2.20 p.m.	5·66
4.30 p.m.	6·25	3.20 p.m.	5·65
5.30 p.m.	6·30	4.20 p.m.	5·82
6.30 p.m.	6·14	5.20 p.m.	5·80
		6.20 p.m.	5·82

In experiments in which food was withheld, the *p*H rose slowly to 7 and over; the highest point recorded in these series was 7·48 after 48 hr. fast. In one experiment the fall in *p*H was marked when the animal was subsequently fed after fasting and the low figure of 4·36 was reached $8\frac{3}{4}$ hr. later; the sheep appeared to suffer no ill effects, and the next morning the *p*H was again within the normal range. Excluding this result the extreme figures recorded in eleven experiments during normal feeding was 6·73–5·33.

These experiments established that the acidity of the rumen varied in a regular manner, and it was decided to investigate the fluctuations in the quantity of the organic acids which are responsible for the fluctuations in *p*H. The sheep were fed for periods of a month or longer on three standard rations, and finally two were studied while grazing freely at pasture. The first ration used was the same as that used during the preliminary experiments. The second was designed to contain soluble sugars and consisted largely of ground mangolds and chopped cabbage, together with chaffed hay and straw and a little bran and oats. The third consisted of hay alone. The following routine procedure was adopted: the manger was cleared of all food on the afternoon preceding the period of observations. The rumen ingesta was sampled before feeding the next morning and at appropriate intervals after feeding. When the animal had eaten as much as it wished, the manger was again cleared and no further food was given until the experimental period was over. Water was available all the time.

Bran and oats diet

In the first few experiments small quantities of lactate were found in the ingesta for 2 hr. or so after feeding; in subsequent experiments, however, only traces or none at all were detectable. These experiments were conducted at intervals over a period of a year, and several months elapsed between the first experiments and the subsequent ones; apparently the balance of the bacterial flora had altered in the intervening period. The following experiment illustrates the type of result obtained in the first few experiments:

Hours after feeding	pH	Lactic acid mg. %
Before	7·36	None
1 hr. after	6·77	38·8
2 ,,	6·65	42·8
3 ,,	6·62	4·0
4 ,,	6·60	Trace
5 ,,	6·55	None

The continued fall in *p*H after the lactic acid had disappeared indicates that lactic acid alone is not responsible for the acidity of the rumen ingesta. In all other experiments the volatile acids also were estimated and the fall in *p*H was, generally speaking, accompanied by a rise in volatile acids.

Hours after feeding	*p*H	Volatile acids c.c. 0·1 *N* %	Lactic acid mg. %
Before	7·06	63·1	None
½ hr. after	6·87	73·8	Trace
1 ,,	6·62	87·3	Trace
2 ,,	6·36	110·9	Trace
4 ,,	6·06	131·1	—
6 ,,	6·09	120·1	—
8 ,,	6·09	122·8	—

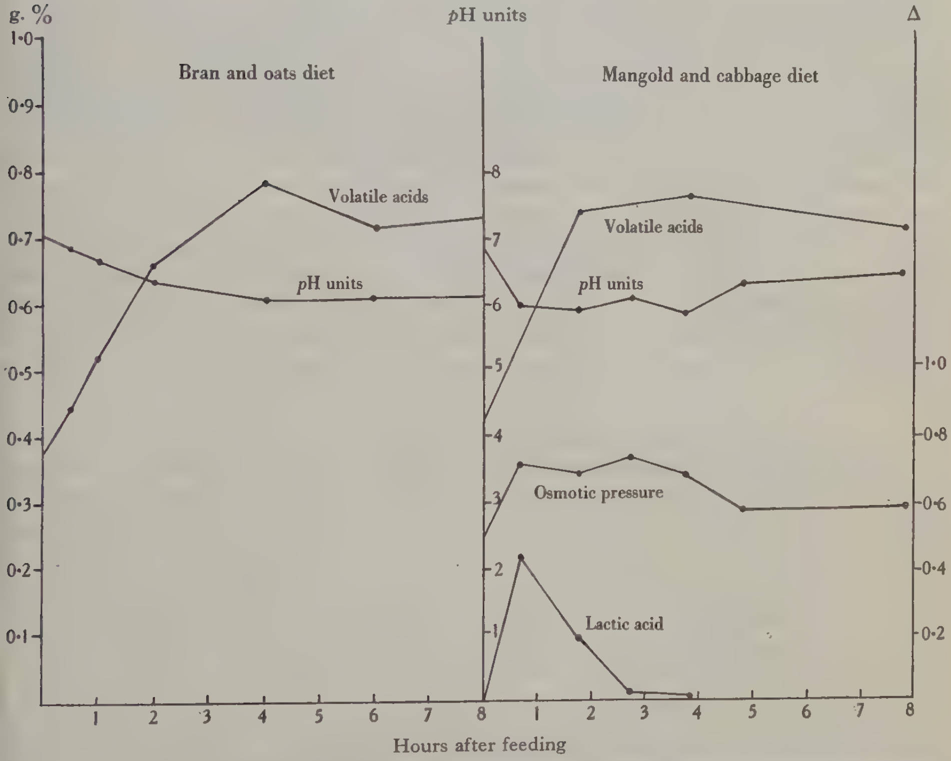

Fig. 1. Chart showing the type of result obtained after feeding the bran and oats diet, left, and the mangold and cabbage diet, right. Volatile acids are expressed as g. % acetic acid. The sheep was fed a few minutes after the first sample was taken.

In this case the highest point of the volatile acids coincided with the lowest point in *p*H. This, however, was not always so, and reference to Table 1 shows that in only three out of eight experiments did the two points occur together. The reason for this is that the lowest point in the *p*H values is not outside the margin of error. Samples taken one after another from the rumen for *p*H measurement gave a variation of 0·14 unit;

for this reason the lowest level of *p*H only is significant, and in estimating this period values are taken that do not differ more than 0·2 unit from the lowest point; thus in the above experiment the period of low *p*H would be 4 hr. onwards. In all experiments the peak of volatile acid curve fell within this low period of *p*H.

The volatile acid figures were also subject to certain variations. In some experiments it was found that a sample taken soon after feeding gave a lower figure than the sample before feeding. This was usually accompanied by a rise in *p*H. This phenomenon was more marked on the hay diet than on the bran and oats diet. The explanation appears to be that the increased flow of saliva due to the stimulation of feeding dilutes the rumen ingesta before any significant fermentation has occurred, and so produces a rise in *p*H as the saliva in ruminants is alkaline, the *p*H being in the region of 8·5 (Lenkeit, 1933).

Other variations were also noticed besides this initial dilution, either in the ascending part of the curve or else in the region of the peak. Similar results were obtained in Quin's laboratory (personal communication from Dr R. A. McAnally) with Merino sheep in which a marked fall was found to occur in the rising curve of volatile acids in some sheep. This occurred either between ½ and 1 hr. after feeding or in others later. It seems probable that this effect was due to the sheep drinking water, and an experiment was performed to see whether this was so. The experiment was conducted in the usual way except that no drinking water was available; volatile acids increased as usual, the curve being rather steeper than normal until 4¾ hr. after feeding, when the high level of 191·7 c.c. 0·1 *N*% volatile acids was reached. At this point the rumen contents were very dry and difficult to sample; water was given and the sheep drank nearly 3 l. before the next sample was taken. This was reflected by a definite drop in the volatile acid content, but the *p*H remained unchanged. The details of the experiments are as follows:

Hours after feeding	*p*H	Volatile acids c.c. 0·1 *N* %
Before	7·17	79·2
½ hr. after	7·03	85·9
1 ,,	—	109·7
1½ ,,	6·73	133·3
2 ,,	6·68	148·3
4½ ,,	5·95	191·1
Sheep drank 2930 c.c. water		
5¼ hr. after	5·96	179·7
Sheep drank 900 c.c. water		
8 hr. after	6·50	169·3

The first dilution with drinking water produced no change in *p*H, while the second dilution, although far less, was accompanied by a rise in *p*H and a slight fall in volatile acid. If the curve of volatile acids is plotted and the drop in volatile acids which occurred when the sheep first drank water is added to the value of the last sample, a smooth curve is obtained with its peak at 5½ hr., in other words the peak of fermentation was over by the eighth hour and the curve was descending; this accounts for the rise in *p*H and not the second dilution that occurred when water was drunk. The conclusion to this experiment is that when the sheep drinks water it passes to the rumen and dilutes the contents so that a drop occurs in the volatile acids, but the *p*H is not affected; consequently if the rate of fermentation is to be determined, both the *p*H and the volatile acid curves must be obtained, as the one without the other is liable to be misleading. The *p*H can be considered only as a general guide to the fluctuation in volatile acids, while a drop in volatile acids, if unaccompanied by a rise in *p*H, is an indication that dilution has occurred through drinking water.

The maximum variations found in eight experiments that are comparable were *p*H 7·12–5·21, and 45·3–143·3 c.c. 0·1 *N* % volatile acids. The lowest period of *p*H extended on an average from 3½ to 8 hr. after feeding, while the average peak in the volatile acids occurred 5½ hr. after feeding.

Effect of fasting

Three sheep were fasted for 48 hr. and all litter was removed from the stalls in order to ensure that they ate nothing during this period. The result was the same in each case; the volatile acids progressively fell and the *p*H steadily rose as occurred in the preliminary experiments. One variation in the otherwise even course of events occurred at the 36th hour of fast (10 p.m. on the second day), when the volatile acids rose and the *p*H fell slightly. This occurred in all three animals and the details of one are given.

Time	Hours of fast	*p*H	Volatile acids c.c. 0·1 *N* %
10 a.m.	24	6·86	76·9
2 p.m.	28	6·99	64·9
6 p.m.	32	7·02	55·9
10 p.m.	36	6·95	59·4
2 a.m.	40	7·14	47·0
6 a.m.	44	7·30	37·7
10 a.m.	48	7·49	32·7

The rise in *p*H is admittedly not outside the margin of error, but this irregularity occurring in all three animals at the same time does not suggest that this observation is due to experimental error. The absence of any reason why fermentation should be accelerated at this period leads to the supposition that the rumen ingesta became concentrated, possibly due to a rapid passage of the liquid part of the ingesta to the abomasum, so that the bacteria which are in intimate contact with the ingesta were also concentrated and their effects became more marked.

The steady fall in volatile acids indicated that fermentation was subsiding, but by no means over; presumably the *p*H of the ingesta would continue to rise until no more volatile acids were being formed when it would be in the region of that of saliva. In the other two sheep the *p*H at the 48th hour of fast was 7·51 and 7·60, while the volatile acid values were 19·9 and 15·1 c.c. 0·1 *N* % respectively.

Mangold and cabbage diet

The characteristic effect of this diet was the large quantity of lactic acid that accumulated temporarily within an hour or so of feeding, but again disappeared from the rumen in a short time. The rapid production of lactic acid was always accompanied by a rapid fall in *p*H; the *p*H, however, remained at a low level after the lactic acid had disappeared and did not rise again until the peak of volatile acid production which succeeded the disappearance of lactate was passed. The following example is typical of the results obtained:

Hours after feeding	*p*H	Volatile acids c.c. 0·1 *N* %	Lactic acid mg. %	Osmotic pressure Δ
Before	6·83	70·6	None	0·505
50 min. after	6·01	—	219·2	0·715
1 hr. 50 min. after	5·98	125·6	98·5	0·690
2 hr. 50 min. after	6·11	—	17·2	0·740
3 hr. 50 min. after	5·94	128·8	6·8	0·688
4 hr. 50 min. after	6·42	—	6·3	0·570
7 hr. 50 min. after	6·46	120·3	Trace	0·580

The osmotic pressure was measured by determining the depression in the freezing-point in this and a few other experiments in order to see if evidence could be obtained of any substance other than organic acids passing into solution during fermentation. The period of maximum osmotic pressure coincided with the period during which the *p*H was at its lowest level, in fact the *p*H and the osmotic pressure, generally speaking, varied inversely with each other; this is good evidence that the organic acids are the main products of fermentation that go into solution.

In the six experiments performed with this diet, the extremes found for *p*H were 7·26–5·94, and for volatile acids 43·2–143·8 c.c. 0·1 *N* %. The period during which the *p*H remained at its lowest level was from 2¼ to 4½ hr., while the volatile acids reached their peak on an average 3¾ hr. after feeding. The lactate reached its peak on an average 1 hr. 20 min. after feeding. Fermentation was quicker than on the bran and oats diet, and a comparison of the two diets is made in Fig. 1.

Hay diet

Results obtained when the sheep were fed on hay alone were variable, in that the average level of volatile acids was different in individual animals. In each experiment, however, the fluctuation was not great and the changes extended over a long period; it was difficult to estimate when the peak of fermentation occurred, as the irregularities due to initial dilution by saliva and subsequent dilutions by drinking water obscured the small changes in acidity. In addition to five experiments in which the rumen was sampled at intervals throughout the day, each sheep was sampled first thing in the morning and last thing in the evening over a period of days in order to obtain more data concerning the extreme fluctuations. The following experiment gave the clearest result:

Time	Sheep fed at 10.30 a.m. Hours after feeding	*p*H	Volatile acids c.c. 0·1 *N* %
10.00 a.m.	Before	7·04	49·4
1.45 p.m.	3¼ hr. after	6·86	52·6
4.30 p.m.	6 hr. after	6·70	60·9
7.00 p.m.	8½ hr. after	6·73	65·2
10.20 p.m.	11 hr. 50 min. after	6·40	80·9
10.40 a.m.	24 hr. 10 min. after	6·89	55·9

Twelve hours after feeding the volatile acids were still rising and the *p*H still falling, consequently it can be said that the fermentation was still increasing; in only one animal did a definite peak occur earlier, when in two experiments it was found to occur in the region of 8 hr. after feeding. In this animal the volatile acid fluctuated at a higher level than in any of the others. Reference to Table 3 shows the general level of volatile acids and *p*H for each animal; unfortunately, in two experiments the *p*H was not determined, but, as the variation was slight in the others, it is doubtful whether any further information would have been obtained if these measurements had been included. In sheep 1, in which the volatile acids were high, the *p*H ranged from 6·75 to 6·04 and the volatile acids from 77·1 to 128·8 c.c. 0·1 *N* %; with sheep 2 the *p*H varied from 7·04 to 6·40 and the volatile acids from 49·4 to 80·9 c.c. 0·1 *N* %, while with the two sheep with the double fistulae the volatile acids varied from 22·4 to 61·8 c.c. 0·1 *N* %. The characteristic feature of these experiments was that fermentation proceeded slowly, and the increase in acidity was comparatively small and spread out over a period that in most cases exceeded 12 hr. No lactate was found in any sample.

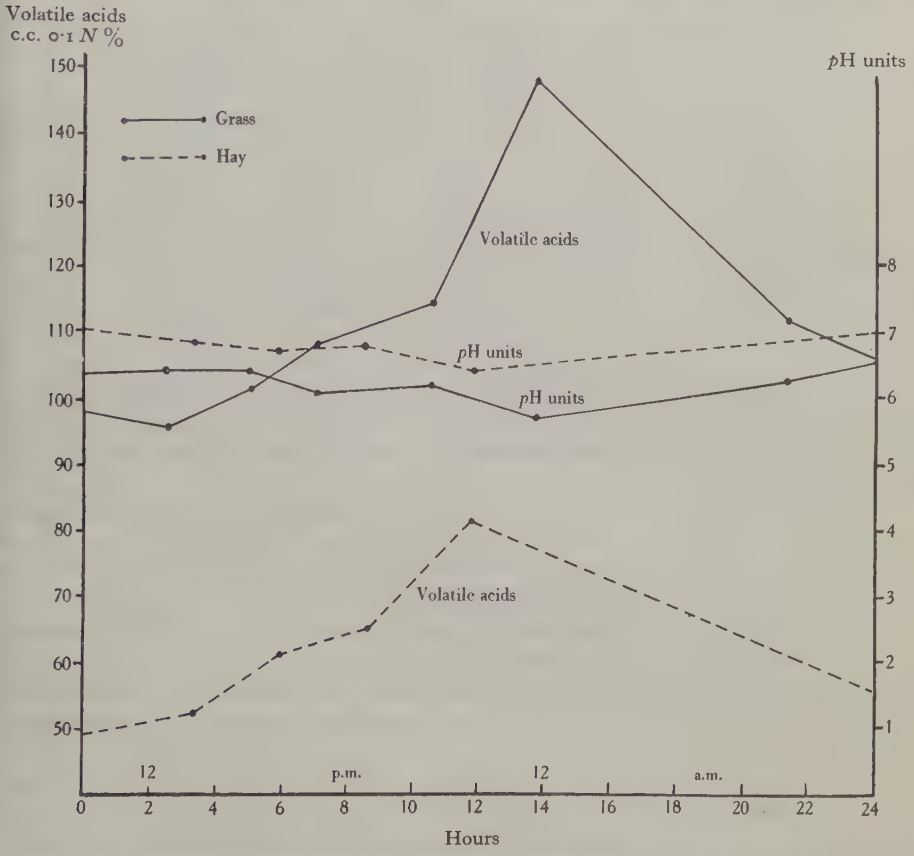

Fig. 2. Chart showing the type of result obtained after feeding hay and while the sheep were grazing. The acidity of the rumen of the sheep at grass is approximately twice that of the sheep fed on hay.

Pasture grass

Two Dorset Horn ewes were studied on the day they were turned out to grass early in May and on two occasions later. The volatile acids rose steadily on the day they were first at grass and reached in one case the high level of 190·4 c.c. 0·1 *N* % $6\frac{3}{4}$ hr. later.

This high level was maintained; samples taken throughout the day after they had been at grass for a week and again throughout the 24 hr. when they had been out a fortnight showed that volatile acids fluctuated at a higher level and the *p*H varied at a lower level than on any of the stall-fed diets. The feeding habits at grass were more extended, consequently it is not possible to estimate the peak of fermentation after any one feed. The results obtained from both ewes after they had been at grass for two weeks are given below.

Time	Sheep 3		Sheep 4	
	*p*H	Volatile acids c.c. 0·1 *N* %	*p*H	Volatile acids c.c. 0·1 *N* %
10.00 a.m.	6·37	98·3	6·25	112·0
12.15 p.m.	6·45	95·8	6·17	136·7
2.30 p.m.	6·44	101·2	6·24	126·6
5.00 p.m.	6·06	107·8	5·94	104·6
8.30 p.m.	6·24	114·9	5·96	151·5
11.45 p.m.	5·71	147·1	6·08	162·4
6.30 a.m.	6·25	111·9	6·42	131·4
10.00 a.m.	6·54	106·4	6·39	121·2
12.45 p.m.	6·63	—	6·32	—
5.45 p.m.	6·31	—	6·35	—

In sheep 3 the lowest point occurred at midday and the highest at midnight, while in sheep 4 two distinct waves occurred, a small rise in the morning reaching its peak at midday and a large rise in the evening reaching its peak at midnight. The times indicated are 2 hr. ahead of solar time.

On the days in which the animals were turned out to grass the peak occurred in one sheep 4½ hr. later, while in the other the volatile acids were still rising 6½ hr. afterwards. An experiment covering 24 hr. done on a ewe fed with cut grass and hay gave values which were within the range found for sheep at grass, volatile acids varying from 93·7 to 162·1 c.c. 0·1 *N* %; the peak in this case occurred 7½ hr. after feeding. Table 4 gives the figures for all experiments with the sheep at grass. These experiments were similar to those performed when the sheep were fed hay in that the changes were spread over a long period, but the differences between the extremes were greater, as is illustrated by Fig. 2.

DISCUSSION

Since this work was started three reports concerning the fluctuation of *p*H of the rumen ingesta in cattle have been published (Monroe & Perkins, 1939; Hale, Duncan & Huffman, 1940; Smith, 1941). The average figures found by Monroe & Perkins are slightly higher than those reported here; when grain and hay, or silage and grain or straw alone were fed average figures ranged from 6·83 to 7·14; lower values were found, however, when the animals were grazing, in agreement with the results obtained here from sheep. On the other hand, Smith, who measured the *p*H with a glass electrode in vivo, found lower average figures, namely, 6·27 with alfalfa hay alone and 6·00 when alfalfa hay and beet pulp were fed. Except for the slight difference in average values, which are hardly large enough to be of much significance, the *p*H of the rumen ingesta in cattle seems to behave in much the same manner as it does in sheep.

The variations in the values found for Δ, 0·505–0·740, cover the figures given by

Davey (1936), who found the value of Δ of the rumen liquid of two 4-month-old lambs to be 0·625 and 0·630.

A comparison of the median points between the average extremes of *p*H and volatile acids given in Tables 1–4 shows that the ingesta of the rumen was most acid while the sheep were at grass and least acid while they were fed on hay alone.

Diet	Mid-point between average variation of *p*H	Mid-point between average variations of volatile acids c.c. 0·1 *N* %
Bran and oats	6·47	91·9
Mangold and cabbage	6·60	90·1
Hay	6·50	75·9
Grass	6·09	140·5

The probable explanation why young pasture grass produces a greater acidity in the rumen is that the plant tissues are more easily attacked by bacteria and because sheep at pasture eat larger quantities of grass than they do when on a dry diet. Woodman, Evans & Eden (1937) measured the appetites of sheep at grass and of sheep kept on a diet of hay, concentrates and swedes and found that sheep at pasture ate 1–1½ lb. more dry matter per week than they would have done if fed on the other diet. As the rumen contains more food then, it is reasonable to expect greater fermentation.

The type of carbohydrate affects the rate of fermentation; the peak of the volatile acids and the low period of *p*H occurred soon after feeding when mangolds and cabbages were fed, it was delayed with bran and oats, and on a hay diet it occurred in most animals 12 hr. or more after feeding.

Diet	Average period of low *p*H	Peak of volatile acids
Mangold and cabbage	2½ to 4½ hr. after feeding	3¾ hr.
Bran and oats	3½ to 8 hr. after feeding	5½ hr.
Hay	—	12 hr. or more; in one case 8 hr.

Little information is available in the literature regarding the rate of fermentation in the rumen; Rathnow (1938) used the iron contained in the food as an index of digestion and found that in a sheep with a rumen fistula 32% of the digestible part of hay passed into solution in 3 hr., 48% in 6 hr. and 59% in 9 hr. after feeding. Hale *et al.* (1940) made a similar study on a cow fed on hay, and on six occasions removed the whole of the rumen ingesta through a fistula 14 hr. after feeding, and using the lignin of the food as an index estimated that 85% of the digestible carbohydrate had disappeared from the rumen in this time. This evidence also indicates that the fermentation of cellulose is not complete within a few hours, but is spread over a comparatively long period.

If the average figures for volatile acids on the various diets are expressed as acetic acid, and assuming that 3 l. of liquid are present in the rumen, it is seen that the quantity in the rumen is considerable:

Bran and oats	16·5 g. acetic acid
Mangold and cabbage	16·2 g. ,,
Hay	13·6 g. ,,
Grass	25·3 g. ,,

Table 1. *Bran and oats diet*

Sheep	Variation pH	Median point	Time after feeding of lowest point hr.	Period of lowest region of pH hr.	Variation volatile acids c.c. 0·1 N %	Median point	Time after feeding of highest point hr.
2	6·69–6·23	6·46	8	2 –8	95·4–131·2	113·3	8
1	7·12–5·65	6·38	12	6 –12	54·2–143·3	98·7	6
2	7·12–5·96	6·54	5	5 –10	56·7–120·0	88·3	7½
3	7·06–6·20	6·63	7	4½– 7	65·8–108·2	87·0	7
1	7·06–6·06	6·56	4	4 – 8	63·1–131·1	97·1	4
3	7·02–5·85	6·43	4½	3 – 6½	60·6–115·4	88·0	3
4	6·80–6·54	6·00	4½	3 – 4½	77·4–129·1	103·2	3
2	7·10–6·54	6·82	3½	1½– 7½	45·3– 74·4	59·8	5½
Average	6·99–5·96	6·47	6¼	3½– 8	64·8–119·1	91·9	5½

Table 2. *Mangold and cabbage diet*

Sheep	Variation pH	Median point	Time after feeding of lowest point hr.	Period of lowest region of pH hr.	Variation volatile acids c.c. 0·1 N %	Median point	Time after feeding of highest point hr.
1	6·98–6·02	6·50	1¾	1¼–3¾	73·4–143·8	108·6	3¾
3	7·07–6·74	6·90	2½	2½–3½	67·0– 83·9	75·4	2½
4	6·98–5·95	6·46	1¾	1 –3½	68·5–128·9	98·7	3½
2	7·26–6·35	6·80	8	4 –8	46·9–113·7	80·3	5½
1	6·83–5·94	6·36	4	2 –4	70·6–128·8	99·7	4
4	—	—	—	—	43·2–112·7	77·9	3
Average	7·03–6·20	6·60	3½	2¼–4½	61·6–118·6	90·1	3¾

Table 3. *Hay diet*

Sheep	Variation pH	Median point	Time after feeding of lowest point hr.	Period of lowest region of pH hr.	Variation volatile acids c.c. 0·1 N %	Median point	Time after feeding of highest point hr.
1	6·75–6·32	6·53	8	7–12	77·1–106·5	91·8	8
1	6·51–6·04	6·27	12+	6–12	109·9–128·8+	119·3	10
2	7·04–6·40	6·72	12+	12	49·4– 80·9+	65·1	12
6	—	—	—	—	29·2– 69·8	49·5	12
7	—	—	—	—	22·4– 85·1	53·7	12+
Average	6·76–6·25	6·50	—	—	57·6– 94·2	75·9	—

Table 4. *Grass*

Sheep	Variation pH	Median point	Variation volatile acids c.c. 0·1 N %	Median point
3	6·63–5·71	6·17	95·8–147·1	121·4
4	6·42–5·94	6·18	104·6–162·4	133·5
3	6·66–6·26	6·46	115·2–130·3	122·7
4	6·38–5·78	6·08	145·7–194·8	170·2
3	6·26–5·44	5·85	109·8–148·7	129·2
4	6·01–5·69	5·85	141·3–190·4	165·8
Average	6·39–5·80	6·09	118·7–162·3	140·5

This opens the question of the value of the lower fatty acids in nutrition. Ringer & Lusk (1910) found that no glucose was excreted in the urine of phloridzinised dogs after dosing with acetic acid and later Lusk (1921) found that dosing acetic acid produced a rise in metabolism, this was confirmed by Deuel & Milhorat (1928), who also showed that no volatile acid was excreted and so concluded that acetic acid must be rapidly oxidized in the body. A recent report from the Animal Nutrition Laboratory, Adelaide (1939–40), states that over 50% of the combustible energy of straw is used in the increased metabolism that follows its ingestion. The dynamic stimulation that occurs after feeding in ruminants has been discussed by Ritzman & Benedict (1938), who point out that in cattle this is large and may exceed the basal metabolic level by 50–100%. This stimulation is due not to the protein in the food as in man and the dog but to the carbohydrate of the diet, and although at first sight it seems obvious that this must be due to bacterial fermentation in the rumen, they consider that this cannot be the whole explanation, as the same effect is found in the horse and the elephant in which fermentation is delayed until the food reaches the caecum.

Evidence that fatty acids with an odd number of carbon atoms can be converted to carbohydrate has been produced by Eckstein (1933) and Deuel, Butts, Hallman & Cutler (1935), who found that acids such as propionic when fed as sodium salts to rats produced glycogen in the liver while those with an even number of carbon atoms such as butyric did not. Ringer (1912) found in phloridzinised dogs that propionic acid was converted almost quantitatively into sugar which was recovered in the urine, while reports from Adelaide (1939–40) state that the amount of glucose excreted in the urine of phloridzinised sheep increases after feeding cellulose, an observation which may mean that propionic is one of the acids formed as a result of fermentation of cellulose.

A mixture of volatile acids is undoubtedly present in the rumen ingesta, as is shown by the fact that if the percentage rate of distillation of the acids from the rumen is plotted logarithmically a curve is obtained which lies between the straight lines obtained by distilling propionic and acetic acids under identical conditions. All the authorities agree that a mixture of the lower fatty acids is found in the rumen (Ellenberger & Scheunert, 1920; Mangold, 1929; Woodman, 1930; Lenkeit, 1933), yet no figures are given to indicate the relative proportions to each other, although the opinion is expressed that these acids are mainly acetic and butyric. In order to account for the feeding value of cellulose by means of the lower fatty acids alone it must be assumed either that propionic acid is produced in sufficient quantity to account for the digestible energy of cellulose that can be stored in the body or else that acetic and butyric acids, besides forming a supply of ready energy, are also potential sources of carbohydrate.

It is still an open question whether the organisms of the rumen are present in sufficient quantity to form an important end-product of fermentation; should this prove to be the case, then the digestible energy of cellulose which can be stored in the body might well be transported to the host in the form of bacteria and other organisms together with any polysaccharide contained in their protoplasm. The matter can only be decided by further research.

SUMMARY

1. The pH of the ingesta of the rumen varies in a regular manner; the speed and extent of the variation depends upon the diet.

2. The changes of pH reflect the fluctuations in the quantities of organic acids that accumulate in the ingesta as a result of fermentation of food in the rumen.

3. Lactic acid accumulates temporarily in the rumen when mangolds and cabbage are included in the ration. The production of lactic acid is accompanied by an abrupt and marked fall of pH.

4. Volatile acids are constantly present in the rumen ingesta; they increase in quantity after feeding and decrease with fasting.

5. The rate of fermentation in the rumen depends upon the diet; it is most rapid when mangolds and cabbage are fed in addition to roughage, less rapid when bran and oats form the concentrated part of the ration, and least rapid when hay alone is fed.

6. The ingesta of the rumen are more acid when sheep are grazing on young pasture grass than when they are stall fed,

7. The osmotic pressure of the ingesta of the rumen varies inversely with the pH.

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STUDIES ON THE FATE OF CARBOHYDRATES IN THE RUMEN OF THE SHEEP

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It was shown in a previous paper (Phillipson, 1942) that the rate of fermentation in the rumen of the sheep appeared to be correlated to the type of carbohydrate present, that a feed of mangolds which contain a high content of soluble sugar, mostly cane sugar, fermented rapidly, that a feed of hay which contains mostly cellulose fermented slowly, whilst starchy foods fermented less rapidly.

In this present paper an account is given of experiments planned to investigate:

(1) the rate of fermentation of specific carbohydrates in the rumen,
(2) the products of fermentation in each case,
(3) the fate of the products formed.

As regards rate of fermentation, it proved that the carbohydrates fell into three classes which are typified by (1) glucose, (2) galactose, (3) cellulose. The behaviour of the *p*H of the ingesta is characteristic for each group.

METHODS AND DOSAGE

Sheep with permanent rumen fistulae or with both rumen and abomasal fistulae were used (Phillipson & Innes, 1939). Food was withheld overnight and samples were taken from the rumen before and at appropriate intervals after dosing. A standard dose of 100 g. was used except with cellulose which was given in the form of filter-paper pulp; of this a dose of 100 g. was too bulky and smaller quantities were given.

ANALYTICAL

The same analytical methods were used as in the previous work (Phillipson, 1942). In many experiments large quantities of lactic acid accumulated in the ingesta, and as this acid is partially volatile, tests were made to find the error caused by this factor in estimating the volatile lower fatty acids. The distilling flask used consisted of a 300 c.c. Kjeldahl flask fitted with a double-bulb splash head; no lactate was found in the distillates and therefore it can be assumed that no error in the estimates for volatile acids occurred from contamination with lactic acid.

Glucose in the ingesta was estimated after suitable dilution by the copper reduction method described by King, Haslewood & Delory (1937) for blood. Sodium was estimated by the pyro-antimonate micro-titration method of Kramer and Gittleman described by Peters & Van Slyke (1932).

RESULTS

A few preliminary experiments in which the *p*H only was measured showed that after administering 100 g. of glucose or cane sugar a rapid fall in *p*H occurred during the following hour; fermentation was intense, judging by the quantity of gas that escaped from the cannula when the stopper was removed and by the frothing of the ingesta. No such reaction occurred after the administration of starch or cellulose; in the former case a slight but steady fall in *p*H was found which continued throughout the day, while with cellulose little change was seen. These observations were subsequently confirmed when full analyses were done.

The effect of introducing large doses of various sugars, starch and cellulose into the rumen

The results can be divided into three groups, those in which intense fermentation occurred characterized by the accumulation of large quantities of lactic acid, those in which fermentation was less marked and in which no lactic acid or only small quantities were found and those in which fermentation was prolonged and in which no violent fluctuations occurred.

Intense fermentation was produced by dosing glucose, fructose and cane sugar; lactic acid accumulated quickly and was accompanied by a marked fall in *p*H; larger quantities of lactic acid were found after dosing glucose and fructose than after dosing cane sugar, and it persisted for longer in the rumen. Volatile acids increased in all cases, but the rise was slower than that of lactic acid, the peak following that of the lactic acid which was greatest from ½ to 1 hr. after dosing. The following example is typical of the results obtained from the glucose group.

Dose: 100 g. glucose

	*p*H	Volatile acids* as acetic acid g. %		Lactic acid g. %
		A	B	
Before dosing	7·2	0·20	0·20	None
½ hr. after	6·0	0·30	0·31	0·28
1 ,,	5·9	0·32	0·33	0·25
1¾ ,,	6·0	0·33	0·36	0·24
2½ ,,	6·2	0·35	0·39	0·20
4½ ,,	6·7	0·30	0·37	0·02
7 ,,	6·7	0·29	0·39	Trace

* It is usually stated that these acids are mainly acetic and butyric; we have made no analysis to differentiate between the various volatile acids.

The figures for volatile acids are given under two columns A and B. The former are the actual values expressed as grams of acetic acid per cent, while the latter are the values obtained after correcting for the fall in volatile acids that occurs if no dose is given. It was found previously that the quantity of volatile acids in the rumen gradually decreased when food was withheld (Phillipson, 1942). These experiments were commenced 24 hr. after the last feed, and from the 24th to the 48th hour the volatile acids steadily fall except for a slight rise that occurs at the 40th hour. On the basis of the average figures obtained from two sheep, the following figures have been used to correct all values for volatile acids to a stable base line:

Hours	Decrease of volatile acids during fasting	
	As c.c. 0·1 *N* %	As acetic acid g. %
0	0	0
1	2·5	0·015
2	5	0·03
3	7·5	0·045
4	10·0	0·06
5	12·5	0·075
6	15·0	0·09
7	17·5	0·105
8	20·0	0·120
12	16·0	0·096
24	31·2	0·187

These corrections were added to the actual values found and the results obtained which are given under column B for all experiments indicate the true increase in volatile acid that occurred as a result of administration of the substance in question.

The second type of reaction was found after dosing galactose, lactose and maltose. No lactic acid was formed except with maltose when small quantities accumulated in one experiment. The fall in *p*H was slow compared with that which occurred when intense fermentation took place; the lowest point was reached from 4–5 hr. after administering galactose or lactose but earlier after dosing maltose. The rise in volatile acids, generally speaking, was reflected by the change in *p*H, but in some experiments increase in volatile acids persisted after the *p*H had commenced to rise again. This type of response is illustrated by the following experiment.

Dose: 100 *g. galactose*

	*p*H	Volatile acids as acetic acid g. %		Lactic acid g. %
		A	B	
Before dosing	6·7	0·32	0·32	None
½ hr. after	6·7	0·27	0·28	None
1½ ,,	6·5	0·35	0·37	None
2½ ,,	6·4	0·38	0·42	None
4½ ,,	6·2	0·42	0·49	—
8 ,,	6·6	0·33	0·45	—
24 ,,	7·1	0·14	0·33	—

The initial fall in total volatile acids after dosing was found in many experiments and is presumably due to dilution of the ingesta although the water in which the sugar was dissolved was kept as small as possible; stimulation of the salivary flow as a result of dosing is also a possible explanation although the fact that no rise in *p*H occurred does not suggest that this was marked.

The last type of reaction was obtained only after dosing starch or cellulose. In both cases the fall in *p*H and the rise in volatile acids was slight but prolonged. In some experiments the starch was baked before administration in order to split up the granules but this did not produce a greater response.

Dose: 100 g. baked starch

	pH	Volatile acids as acetic acid g. %	
		A	B
Before dosing	7·1	0·37	0·37
2 hr. after	6·9	0·37	0·40
4 "	6·9	0·39	0·45
6 "	6·8	0·41	0·50
8 "	6·8	0·41	0·53
24 "	7·3	0·23	0·42

Dose: 30 g. filter-paper pulp

	pH	Volatile acids as acetic acid g. %	
		A	B
Before dosing	7·2	0·30	0·30
1 hr. after	7·2	0·25	0·26
4 "	7·1	0·30	0·36
8 "	7·0	0·32	0·44
12 "	7·0	0·34	0·43
24 "	7·2	0·25	0·44

These two experiments are quoted as they show the maximum change observed after dosing starch and cellulose respectively; in both the rise in volatile acids is prolonged and the corrected figures under column B indicate that the fermentation of starch proceeds less slowly than the fermentation of cellulose.

The results of the experiments with sugars agree with those obtained by McAnally (in Press) who studied the fermentation of carbohydrates by liquid rumen contents in vitro. She estimated the rate of fermentation by the evolution of gas and found that glucose was fermented quickly; appreciable quantities of gas accumulated after 10 min. incubation and steadily increased during the 90 min. period of observation. Cane sugar behaved in much the same way except that a slight initial lag occurred. Fermentation of maltose was slow in comparison but proceeded more quickly in the later stages, while little fermentation at all was obtained with lactose.

The fate of glucose, lactic acid and volatile acids

The fate of glucose was investigated in greater detail as it has been suggested that it forms the product of cellulose fermentation that can be stored in the body (Woodman, 1930). This involved the study of the rates of appearance and disappearance of lactic acid and of the volatile acids as both are produced when glucose is introduced into the rumen. The results are best described under the headings of the substance in question, and to do this it is necessary to split up the experimental results; each experiment in which glucose was dosed involved estimations of all three substances and the data are too bulky to discuss in any other way.

Glucose

The disappearance of glucose from the rumen after dosing 100 g. was followed in four experiments; in one experiment glucose had disappeared in 2½ hr., and in the other three it had disappeared in from 1½ to 2 hr. after administration. In these three experiments abomasal ingesta were taken as well; in one, no trace of any reducing substance could be found in any one of the samples taken, while in the other two experiments only relatively small amounts were found in the abomasal ingesta. The details of two of these experiments are as follows:

Dose: 100 g. glucose, administered directly into the rumen

	Rumen, glucose mg. %	Abomasum, glucose mg. %
1. Before dosing	None	None
½ hr. after	306	32
1 ,,	23	16
1½ ,,	9	11
2 ,,	None	None
2. Before dosing	None	None
¼ hr. after	552	None
¾ ,,	43	None
1¾ ,,	None	None

It is obvious that the rapid disappearance of glucose from the rumen cannot be accounted for by passage to the abomasum. The quick appearance of large quantities of lactic acid and the increase of volatile acids which accompanies the disappearance of glucose indicate that fermentation is rapid.

The rate at which glucose disappears in vitro was also investigated. Samples of fluid obtained by filtering the ingesta through muslin fermented glucose rapidly provided excess substrate was not introduced, but the supernatant fluid obtained by centrifuging rumen ingesta possessed little fermentative power, an observation previously made by McAnally (in Press). If too much glucose was added, fermentation commenced rapidly but slowed down after 4 hr. or so; this was due presumably to the accumulation of lactic and volatile acids with a corresponding drop in *p*H. In no case did fermentation proceed as rapidly as in vivo. In the experiment quoted below the substrate was present in excess and complete disappearance did not occur; the rate of fermentation was rapid during the first 4 hr., 0·296 g. % of glucose were fermented in 2 hr., 0·428 g. % in 4 hr., but only 0·496 g. % in 6 hr.

	Beginning of experiment			End of experiment after 6 hr. at 37° C.		
	Glucose g. %	Volatile acids as acetic acid g. %	Lactic acid g. %	Glucose g. %	Volatile acids as acetic acid g. %	Lactic acid g. %
Control flask	None	0·23	0·002	None	0·26	None
Glucose flask	0·70	0·23	0·002	0·21	0·33	0·098

In two other experiments 0·91 and 0·62 g. % of glucose disappeared in 7½ hr. at 37° C., the quantities of lactate formed being 0·148 and 0·366 g. % respectively, while volatile acids expressed as acetic acid increased by 0·18 and 0·13 g. % respectively over the control flasks.

There can be no doubt that glucose is fermented by rumen ingesta and that lactic and volatile acids are produced; the fact that fermentation is slower in vitro than in vivo is only to be expected, as no means of eliminating the products of fermentation are present. The quantity of lactic acid and volatile acids produced was small in comparison with the amount of glucose lost, but as no account was taken of the carbon lost as methane and carbon dioxide, quantitative results were not expected. The fate of glucose in the rumen is clear; it is rapidly fermented and little, if any, passes to the abomasum. It is, however, possible that some is directly absorbed from the rumen or during its passage through the omasum, and this aspect will be discussed later.

Lactic acid

The rapid appearance and disappearance of lactic acid suggests that it is a transitory stage in the fermentation of glucose by bacteria of the rumen, especially as Woodman & Evans (1938) found that mixed cultures of cellulose-splitting organisms from the rumen of the sheep attacked lactate, the fermentation being characterized by the production of volatile fatty acids.

Solutions of sodium lactate were administered into the rumen, and the rate of disappearance together with the increase of volatile acids was estimated. Seven experiments were done and doses ranging up to 25 g. sodium lactate were administered; in all the experiments lactate disappeared from the rumen, in some cases within 2 hr. of dosing and in others within 4½ hr. The quantity of volatile acids produced was variable, irrespective of the size of the dose; in only two experiments was there a marked rise; in the other experiments the level of volatile acids remained more or less constant. An example of each type of result is given, both of which were obtained from the same sheep.

Dose: 12·5 *g. sodium lactate*

	*p*H	Volatile acids as acetic acid g. %		Lactic acid g. %
		A	B	
Before dosing	7·0	0·44	0·44	Trace
¼ hr. after	7·0	0·39	0·40	0·1816
1 ,,	7·1	0·37	0·39	0·0953
2 ,,	7·0	0·40	0·43	0·0201
4 ,,	7·0	0·41	0·47	0·0038
8 ,,	7·1	0·40	0·52	None

Dose: 8·8 *g. sodium lactate*

	*p*H	Volatile acids as acetic acid g. %		Lactic acid g. %
		A	B	
Before dosing	6·9	0·67	0·67	None
¼ hr. after	7·0	0·63	0·64	0·1669
1 ,,	7·0	0·77	0·78	0·0654
1¾ ,,	6·9	0·74	0·77	0·0090
2¾ ,,	7·1	0·76	0·80	Trace
3¾ ,,	6·8	0·84	0·89	—
5 ,,	6·8	0·74	0·81	—

In the first experiment volatile acids fell slightly and the rise in *p*H suggests that this was due in part to an increase in the flow of saliva; although the dose was larger than that given in the other experiment, apparently little of the lactate was converted to volatile acid. On the other hand, when the smaller dose was given the increase in volatile acids was substantial.

In vitro experiments were also performed in which the disappearance of lactate was followed and the volatile acids formed were estimated. These experiments were subject to much variation. The supernatant fluid obtained after centrifuging the rumen ingesta had little activity upon lactate, but the activity of ingesta filtered through muslin exerted a variable effect. It was found, however, that if the amount of sodium lactate introduced was small, disappearance occurred within 5 to 6 hr.; in later experiments lactate was added to the rumen liquid in quantities not exceeding 0·2 g. %. The rate of disappearance under these conditions was comparable to that which occurred in vivo. The effect of introducing sheep saliva (filtered through a Berkfeld filter) into the fermentation flask as a buffer had no favourable effect upon the rate of disappearance of lactate when compared with flasks in which the same quantity of distilled water was added. The loss of lactic acid if expressed in 0·1 *N* % was approximately equal to the increase of total volatile acids expressed in the same way. The following experiment is typical:

Time of incubation	Lactate flask 100 c.c. rumen liquid + 10 c.c. saliva + 1 c.c. lactate solution, containing 0·2258 g. lactic acid		Control flask 100 c.c. rumen liquid + 11 c.c. water	
	Volatile acids acetic acid g. %	Lactic acid g. %	Volatile acids acetic acid g. %	Lactic acid g. %
Start	0·56	0·189	0·56	0·0038
5 min.	—	0·175	—	—
2 hr.	—	0·083	—	—
4 hr.	—	0·028	—	—
6 hr.	0·83	0·006	0·66	None

In this experiment a flask containing 100 c.c. of rumen liquid, 10 c.c. of water and 1 c.c. of lactate solution was included; the result in this flask was almost exactly the same as that found in the flask containing saliva. The lactate flask lost 0·183 g. % lactic acid and gained 0·17 g. % volatile acids expressed as acetic acid over the control flask. These figures given in c.c. 0·1 *N* % show that the loss of lactate is accounted for by the gain in volatile acid; the lactic acid lost is equivalent to 20 c.c. 0·1 *N* % and the volatile acid gained to 28 c.c. 0·1 *N* %. The close correlation of the loss of lactate and gain in volatile acids suggests that the reaction proceeds molecule for molecule, and that the probable acid formed is acetic or propionic. Similar results were obtained in two other experiments.

As lactate may persist in the rumen for 4 hr., samples were taken from the abomasum in two experiments after dosing lactate into the rumen and in two experiments after dosing glucose into the rumen. Appreciable amounts of lactate appeared in the abomasum after dosing 36·3 g. sodium lactate, but only traces appeared after administering half this dose into the rumen. The quantity of lactic acid appearing in the abomasum after dosing

100 g. glucose into rumen also depended upon the concentration of lactic acid reached in the rumen. The details of these experiments are as follows:

	Rumen lactic acid mg. %	Abomasum lactic acid mg. %
Dose: 36·3 g. sodium lactate:		
Before dosing	None	None
20 min. after	464·4	14·3
1 hr. after	284·8	88·2
2¼ hr. after	54·4	27·1
4½ hr. after	5·1	None
Dose: 18·1 g. sodium lactate:		
Before dosing	None	None
25 min. after	115·2	1·5
1 hr. after	91·9	None
1 hr. 50 min. after	54·5	None
3 hr. 50 min. after	14·3	None
Dose: 100 g. glucose:		
Before dosing	Trace	None
¼ hr. after	174·6	7·1
¾ hr. after	260·1	33·7
1¼ hr. after	274·6	36·5
1¾ hr. after	178·0	16·1
2¼ hr. after	89·0	4·2
4 hr. after	None	None
Dose: 100 g. glucose		
Before dosing	21·2	5·8
¼ hr. after	254·7	4·5
40 min. after	423·0	54·0
1 hr. 5 min. after	420·3	91·4
1 hr. 35 min. after	210·6	62·1
2 hr. 5 min. after	51·7	18·0
2 hr. 35 min. after	18·0	13·5
4 hr. 5 min. after	Trace	Trace

The rate of disappearance of lactate in these sheep with double fistulae was slower than in the other animals, consequently the chances of lactate appearing in the abomasal ingesta were increased; even so, the quantity found was not large in comparison with the amount found in the rumen. In the latter two experiments the quantity of glucose passing to the abomasum was estimated, in the first no glucose appeared while in the second a little was found 15 min. after dosing but at no other time.

The fate of lactate therefore is similar to the fate of glucose except that it does not appear to be so rapidly fermented in vivo, consequently a larger proportion passes to the abomasum. The concentration of lactic acid in the abomasum never approaches that of the rumen, and as the volume of the abomasal contents is approximately one-tenth that of the rumen the loss of lactate from the rumen cannot be accounted for by passage to the abomasum. Lactic acid is not stable in the rumen ingesta and so its disappearance from the rumen can be attributed in part to fermentation but the possibility of direct absorption must still be considered.

Volatile lower fatty acids

It was found in experiments performed on a sheep with an abomasal fistula only that the quantity of volatile acids present in the abomasal ingesta was small and did not increase significantly after feeding as would be expected from the large quantities formed in the rumen. The animal was fed on bran and oats together with chaffed hay and straw, a diet known to increase the concentration of volatile acid in the rumen by 100% or over in from 4 to 8 hr. The following experiment illustrates the results obtained:

Feed: bran and oats with chaffed hay and straw

	*p*H	Titratable acid c.c. 0·1 *N* %	Volatile acid* c.c. 0·1 *N* %
Before	2·91	38·4	6·6
2 hr. after	2·87	39·9	11·9
4 "	2·89	41·0	13·1
6 "	2·96	41·2	12·1
8 "	2·84	42·6	12·3

* The distillates were tested with silver nitrate but no chloride was found.

Additional proof that little volatile acid was present in the abomasal ingesta was obtained by estimating the active chloride; it was found that the greater part of the titratable acid could be accounted for by gastric secretion, and consequently that only small proportions of the titratable acidity, often less than those found in the above experiment, were due to organic acids. As the volatile acids are considered to be an end-product of carbohydrate fermentation in the rumen, and as the concentration of these acids present in the rumen is considerable, ten or more times the concentration found in the abomasum, it was decided to investigate the fate of acetic and butyric acids when introduced into the rumen.

The acids were given as the crystalline sodium salts, and a standard dose of 50 g. was used. Five experiments were done in which acetate was administered and two in which butyrate was given. In three experiments the sodium of the rumen and abomasal contents was also followed in order to see whether this behaved in the same manner as the acid radicle. Two control experiments were also performed in which it was found that an increased concentration of sodium occurred in the abomasal ingesta after adding 200 c.c. of water to the rumen, consequently the abomasal analyses for sodium after dosing sodium salts of the volatile acids are of no value. The results of these experiments are given in detail in Table 1.

It will be seen that in two of the experiments in which acetate was administered the results were disappointing; in Exp. 1 only a slight rise in volatile acids occurred after dosing, while in Exp. 3 the figures were extremely irregular. In the other experiments and in those in which butyrate was given, there was a marked rise of volatile acids which in two cases did not reach its peak until 1 hr. after dosing; the concentration did not remain high but rapidly declined until by the sixth hour it had reached the region of the predosing level.

In two of the three experiments in which the sodium in the rumen was followed, the initial rise in sodium was proportional to the rise in volatile acids, and the subsequent decline in concentration followed that of the volatile acids reasonably closely, but in Exp. 4 the maximum concentration of sodium preceded that of the acetate and the subsequent decline was not maintained. The irregularity in this experiment may be due to the saliva which contains sodium in a concentration similar to that of blood, namely, in the region of 300 mg. %, consequently an increase in the inflow of saliva to the rumen would increase the concentration of sodium in the ingesta.

In no experiment did volatile acid reach the abomasum in any quantity; the slight increase in concentration that occurred in some experiments was insignificant.

These experiments established two main facts; the first is that the increased concentration of volatile acids produced in the rumen by administering acetate or butyrate is not maintained and the fall in concentration is rapid compared to that found during fasting (Phillipson, 1942). The second is that only traces of volatile acids are present in the abomasal contents and little or no increase is produced either by feeding or administering acetate or butyrate into the rumen. The possibility that the volatile acids

Table 1. *Experiments in which* 50 *g. of sodium acetate was administered*

	Rumen, volatile acid c.c. 0·1 N %		Abomasum, volatile acid c.c. 0·1 N %
1. Before dosing	46·9		None
¼ hr. after dosing	73·8		—
1¼ ,, ,,	78·8		—
3¼ ,, ,,	56·8		—
5¼ ,, ,,	52·1		—
7¼ ,, ,,	58·6		—
2. Before dosing	74·7		6·7
¼ hr. after dosing	94·7		—
1 ,, ,,	167·2		4·5
2 ,, ,,	89·2		4·5
3 ,, ,,	108·7		4·2
4½ ,, ,,	66·2		1·2
6 ,, ,,	56·9		2·2
7½ ,, ,,	34·8		1·5
3. Before dosing	103·2		6·42
¼ hr. after dosing	95·9		—
1 ,, ,,	112·7		7·51
2 ,, ,,	76·2		6·59
4 hr. 10 min. after dosing	87·8		10·76
6 hr. after dosing	71·1		2·33
		Sodium c.c. 0·1 N %	
4. Before dosing	87·1	94·3	5·5
¼ hr. after dosing	122·4	143·0	—
1 ,, ,,	139·1	128·2	9·0
2 ,, ,,	106·5	119·0	5·9
4 ,, ,,	71·5	—	3·6
6 ,, ,,	61·2	123·7	1·7
5. Before dosing	48·6	101	2·9
¼ hr. after dosing	124·9	167·4	—
1 ,, ,,	96·4	143·2	3·5
2 hr. 10 min. after dosing	79·3	126·0	6·6
4 hr. 30 min. after dosing	66·3	128·7	1·4
6½ hr. after dosing	52·8	111·6	—
Experiments in which 50 g. sodium butyrate was administered			
6. Before dosing	46·6	109·1	3·1
¼ hr. after dosing	113·8	157·3	—
1 ,, ,,	124·6	183·4	1·7
2 ,, ,,	101·9	165·1	0·5
3 ,, ,,	77·5	153·4	1·4
4½ ,, ,,	70·0	150·0	0·8
6½ ,, ,,	63·8	141·3	1·4
7. Before dosing	47·5		6·0
¼ hr. after dosing	354·4		—
1 ,, ,,	126·0		8·2
2 ,, ,,	106·6		6·1
3 ,, ,,	91·6		5·5
4½ ,, ,,	67·7		4·9
6½ ,, ,,	49·6		4·0

are unstable in rumen ingesta had still to be investigated, and to do this five experiments were performed in vitro; in the first, 100 c.c. of liquid rumen ingesta was placed in each of four flasks; to one was added 10 c.c. of water and to the others were added 10 c.c. of a known solution of either sodium acetate or propionate or butyrate. Precautions were taken to keep the rumen ingesta warm. The flasks were incubated for 8½ hr. at 38° C., and at the end of this time the total volatile acids in each flask was determined by steam distillation. The result of this experiment showed that no loss of volatile acid occurred.

Volatile acidity after 8½ hr. incubation at 38° C.

	Volatile acids of control flask plus quantity of acid added	Total volatile acids found by distillation
Acetate flask	161·2 c.c. 0·1 *N* %	173·4 0·1 *N* %
Propionate flask	187·4 c.c. 0·1 *N* %	201·5 0·1 *N* %
Butyrate flask	188·4 c.c. 0·1 *N* %	196·4 0·1 *N* %

Assuming that the increase in volatile acids in each of the three experimental flasks was the same as that occurring in the control flask, then the figures in the left-hand column represent the quantity of volatile acid that should have been present after incubation. The actual quantities determined by steam distillation are given in the right-hand column, and in each flask the amount present was greater than expected; this suggests that fermentation of the existing substrate present in the rumen ingesta was slightly greater in the flasks to which volatile acid was added than in the control flask; there is no evidence that the acids added had been attacked by the bacteria.

In the second experiment four flasks were put up, one as control and the others as experimental flasks; to each of the latter sodium acetate, sodium propionate or sodium butyrate solution was added. A sample of 10 c.c. was taken from each flask before they were placed in the incubator and the total amount of volatile acids present was estimated. The contents of each flask was covered with liquid paraffin to prevent evaporation and further samples were taken after 7½, 24 and 48 hr. incubation. The total volatile acidity was expressed as a percentage and by subtracting the quantity of volatile acids present in the control flask at each period of incubation from the quantity present in the other flasks at the same period, the amount of volatile acid added was obtained. These values are given in the following table:

Difference between volatile acids in the experimental flasks and the control flask at each stage of incubation, expressed as c.c. 0·1 *N* %

Hours of incubation ...	0	7½	24	48
Acetate	57·2	62·9	48·5	−71·0
Propionate	51·1	48·1	25·9	2·3
Butyrate	37·4	39·9	27·1	− 8·3

It will be seen that during the first 7½ hr. the amount of acetate and butyrate apparently increased and propionate slightly decreased, but the changes either way were not marked. The volatile acids were only significantly reduced after 24 hr. incubation, while during the last period the rate of decrease was accelerated to such an extent that a minus figure was obtained with acetate and butyrate, especially the former.

The instability of the volatile acids on prolonged incubation in rumen ingesta in no way invalidates the fact that they were stable for 7½ hr.; the succeeding decrease, which became more rapid with time, indicates that a rapid multiplication of acid-splitting bacteria was occurring. The initial period in which the volatile acids are stable indicates that acid-splitting organisms are normally numerically insignificant in the rumen and only gain preponderance under in vitro conditions.

The results of these experiments were confirmed by three further trials. Approximately normal solutions of sodium acetate, propionate and butyrate were made up and the strengths of their solutions were determined by steam distillation. Different amounts were used in each experiment, but in no case was the concentration of total volatile acids in the flasks raised above 150 c.c. 0·1 *N* %, a figure that is within the range found normally in the rumen ingesta. A sample was taken from each flask after it was put up and the quantity of volatile acids present was determined; in addition, in two of the experiments a sample of the rumen liquid, equal in quantity to that put into the flasks, was distilled; thus the total quantity of volatile acids introduced was estimated twice. The contents of the flasks were covered by liquid paraffin and incubated for 8–8½ hr.; a further sample was taken and the volatile acids present after incubation were estimated. The results are expressed as the total volatile acids present in the flask in c.c. 0·1 *N* % assuming that none of the contents were removed during sampling. The full results of the three experiments are given in Table 2.

Table 2

	Before incubation				After incubation		
	V.A. estimated to be added to each flask		V.A. estimated by means of an aliquot		V.A. estimated by means of an aliquot		Difference between V.A. added before and after
	Total V.A.	V.A. added	Total V.A.	V.A. added	Total V.A.	V.A. added	
Exp. 1. Each flask contains 25 c.c.:							
1. Control flask	8·6	—	8·7	—	12·3	—	—
2. Acetate flask	33·8	25·2	33·0	24·2	35·8	23·5	−0·7
3. Propionate flask	34·9	26·2	37·5	28·7	35·8	23·5	−5·1
4. Butyrate flask	32·7	24·1	33·2	24·4	39·4	27·1	+2·7
Exp. 2. Each flask contains 30 c.c.:							
1. Control flask	—	—	13·5	—	15·0	—	—
2. Acetate flask	—	20·2	31·9	18·4	35·5	20·5	+2·1
3. Propionate flask	—	21·4	34·3	20·8	36·1	21·1	−0·2
4. Butyrate flask	—	19·3	32·3	18·8	33·3	18·3	−0·6
Exp. 3. Each flask contains 30 c.c.:							
1. Control flask	18·7	—	18·0	—	21·2	—	—
2. Acetate flask	28·8	10·1	27·2	9·2	28·5	7·3	−1·9
3. Propionate flask	29·4	10·7	28·2	10·2	31·5	10·3	+0·1
4. Butyrate flask	28·4	9·7	28·8	10·8	30·4	9·2	−1·6

It will be seen that the results are fairly regular and no significant loss occurred in any experiment. The variations shown in the last column are better expressed as percentages of the amount of acid originally added, as the concentration was different in each experiment. Percentage figures are given in the following table together with the corresponding figures for the first two experiments:

Deviation of volatile acid after incubation from the amount originally introduced

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Acetate %	+20	+10	− 3	+11	−20
Propionate %	+16	− 6	−17	− 1	+ 1
Butyrate %	+ 9	+ 7	+11	− 3	−15

The figures deviate from +20% to −20%, and with each salt plus and minus figures of nearly equal amplitude occur. These deviations are too wide to be accounted for by error in distillation and must be due to slightly different rates of fermentation in the experimental flasks. Even if the greatest loss, namely 20%, is considered alone, this is insufficient to account for the rapid disappearance of acid from the rumen. On the basis of these experiments there is no reason to suppose that acetate, propionate and butyrate are readily broken down by the bacteria of the rumen as are lactate and glucose; in fact, they appear to be sufficiently stable in rumen ingesta to warrant the conclusion that the rapid disappearance from the rumen of volatile acid added in excess of the quantity present is not due to bacterial activity.

The fate of volatile acids appears to be that they are absorbed before they reach the abomasum; this conclusion rests upon the circumstantial evidence that they rapidly disappear from the rumen when introduced in quantites in excess of that already present, that they do not pass to the abomasum and that they are stable for 8 hr. when incubated in vitro in rumen ingesta. If the conception that absorption can occur readily in the rumen or omasum is accepted, then the fact that the volatile acids are always present in the rumen is due not so much to the fact that they are end-products of fermentation but to the fact that they are continually being produced. The rate of production during fasting, when the level of volatile acids is steadily falling, is sufficient therefore only to prevent a rapid disappearance of volatile acid from the rumen and not to maintain a steady level. Support is given to this conception by an experiment in which 25 g. lactic acid was introduced into the rumen. The *p*H of the ingesta fell abruptly to 4·7 and the volatile acids disappeared within 15 min. of dosing; this suggests that the fall in *p*H either killed or temporarily inactivated the cellulose-splitting bacteria so that the supply of volatile acid failed and the acids already present were rapidly absorbed.

DISCUSSION

The difference in the rate of fermentation of sugars in the rumen can be correlated with sugars occurring naturally in foodstuffs. Cane sugar is present in mangolds while glucose and fructose are primary cleavage products. The smaller quantity of lactic acid that accumulated when cane sugar was introduced into the rumen, compared with the amount that was found after dosing glucose and fructose, suggests that this is due to a slight lag while the disaccharide is being hydrolysed to produce glucose and fructose. Lactose and galactose do not occur naturally in the rumen; in suckling lambs milk passes directly to the abomasum and does not enter the rumen (Phillipson, 1939), consequently the slow rate of fermentation is not surprising. It is possible that lactic acid is also a stage in the fermentation of these sugars, but as the process is slow it is further broken down as fast as it is formed and never accumulates. As lactate may appear during the fermentation of maltose which proceeds at a greater speed than that of lactose, this theory has some support.

The ciliates of the rumen must be considered in relation to the fermentation of starch. It is easy to demonstrate under the microscope that these organisms ingest starch granules; Becker (1932) stated that lambs which have been rendered free of ciliates, by fasting followed by a dose of 2% copper sulphate, became rather distended with gas in the rumen after eating food containing maize meal; this observation suggests that the ciliates assist the host by ingesting starch, so protecting it from bacterial fermentation while the ciliates themselves, in which the starch is said to be converted to glycogen (Trier, 1926), may be finally digested by the sheep when they reach the abomasum. The sheep used in these experiments all contained a flourishing fauna in the rumen, and the slight reaction observed after dosing starch may be due partly to the ciliates, although as starch is a polysaccharide it cannot be expected to be fermented so readily as sugar. Steinhauf (1921) found that the abomasal contents of sheep developed a distinct blue staining reaction with iodine after eating crushed oats, while Schalk & Amadon (1928) observed in cows with rumen fistulae that foods such as maize meal when eaten passed to the reticulum and that some passed directly through the omasum to the abomasum without entering the rumen; the action of the reticulum, which contracts briskly approximately once a minute and throws its contents backwards to the rumen, does not allow foods such as maize meal to remain long enough in the region of the omasal orifice for more than a portion of the ingesta to take this direct route. Starch, therefore, may be digested in three ways in the ruminant: some may pass directly to the abomasum and undergo normal digestion as in other animals, some is ingested by the ciliates in the rumen and may be conveyed to the host by these organisms in one form or another, while some is subjected to bacterial fermentation. The proportion that is accounted for by each of these three routes remains a subject for research.

There is ample evidence that the fermentation of cellulose is slow; no one has yet demonstrated the presence of a cellulose-splitting enzyme in any of the digestive secretions of any mammal, while the evidence concerning the ciliates of the rumen all points to the fact that these organisms cannot utilize cellulose in any way (Mangold, 1933); digestion of cellulose in the ruminant therefore must be due to bacteria alone. The observations of Ritzman & Benedict (1938), who found in cattle that the production of methane did not cease until 4 days after the last meal when the animals were fed hay, suggests that fermentation of cellulose must persist in the rumen during this period. McAnally (1942) found that filter paper when enclosed in silk bags required 3–4 days for complete digestion to occur in the rumen, although the presence of the silk barrier probably delayed the onset of fermentation; the prolonged reaction obtained in these experiments after dosing filter-paper pulp into the rumen is in agreement with these findings.

The rapid fermentation of glucose in the rumen rules out the possibility of glucose as such being the principal end product of cellulose fermentation; however, glucose might be made available to the host for absorption if, as Baker (1939) suggests, the iodophilic bacteria, besides hydrolysing cellulose, deposit within their protoplasm a glycogen-like substance which represents the condensation of soluble sugars obtained from cellulose. This may be later digested by intestinal amylases and so reach the host as glucose. The hypothesis originated by Pochon (1935) that glucose is formed in the abomasum from cellulose cannot be true, as no glucose can be found in the abomasal ingesta.

The failure to account for the fate of the volatile acids either by passage to the abomasum or by bacterial decomposition suggests that these acids are directly absorbed either in the rumen and reticulum or during their passage through the omasum. If this is possible then it is also possible that the disappearance of glucose and lactate may also be due in part to direct absorption. The rumen and reticulum are generally stated, on anatomical grounds, to be merely storage and fermentation chambers; the epithelium lining these organs is stratified and squamous, and until recently this has been sufficient reason to deter curiosity regarding absorption from these organs. The omasum is an organ particularly suitable for absorption, as the laminae offer a large surface area which in the cow is equal to that presented by the whole small intestine (Colin, 1886). Other evidence is available that absorption in the non-glandular part of the stomach does occur; thus Aggazzotti (1910) found that water and sugars in solution were absorbed from the omasum but to no great extent from the reticulum or abomasum. Trautmann (1933) proved that drugs such as pilocarpine and atropine were rapidly absorbed through the mucosa of the rumen while Lenkeit & Columbus (1935) found that after dosing potassium iodide to sheep and lambs no difference in the rate of excretion of iodine in the urine occurred, although with adult sheep the dose passed to the rumen and in the lambs it passed directly to the abomasum.

These observations, together with the evidence presented in this paper, although it is admittedly negative evidence, are strong enough to warrant a thorough investigation into the question of absorption from the ruminant stomach and such a programme of research is planned.

SUMMARY

1. Glucose, fructose and cane sugar undergo rapid fermentation in the rumen of the sheep and pass through the stage of lactic acid to volatile lower fatty acids. Maltose, lactose and galactose are fermented less rapidly and lactic acid, if it is a stage in the fermentation, does not accumulate in the ingesta. Starch and cellulose are fermented slowly and the production of volatile acids is prolonged.

2. The rapid disappearance of glucose from the rumen cannot be accounted for by passage to the abomasum.

3. Lactic acid is not stable in the rumen; when it is present in quantity, some appears in the abomasum.

4. Volatile acids are stable in the ingesta of the rumen; they do not pass to the abomasum in any quantity and appear to be absorbed in the rumen or omasum.

5. The progress of digestion of carbohydrates in the stomach of the sheep is discussed.

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